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THE EFFECT OF SUPPLEMENTATION OF ORGANIC ACIDS, OLIGOSACCHARIDES AND LACTITOL ON THE DIGESTIBILITY OF AMINO ACIDS AND BACTERIAL POPULATIONS AND METABOLITES IN THE DIGESTIVE TRACT OF EARLY-WEANED PIGS

BY

©

VINCE M. GABERT

A thesis submitted to the Faculty of Graduate Studies and
Research in partial fulfilment of the requirements for
the degree of

MASTER OF SCIENCE

IN

ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING, 1994

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE EFFECT OF SUPPLEMENTATION OF ORGANIC ACIDS, OLIGOSACCHARIDES AND LACTITOL ON THE DIGESTIBILITY OF AMINO ACIDS AND BACTERIAL POPULATIONS AND METABOLITES IN THE DIGESTIVE TRACT OF EARLY-WEANED PIGS submitted by VINCE M. GABERT in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL NUTRITION.

Dr. Willem C. Sauer Supervisor

Michael I. McBurney

Dr. Michael I. McBurney

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Dr. Lech Ozimek

Dated: December 22 1993

We feed our horses hay and oats,

With grass for cows and sheep and goats.

Chickens look for grain to eat,

While ducks find worms, and dogs get meat.

Cats have meat and milk and fish;

To each, its own peculiar dish.

Some are fussy, others not,

But pigs, of course, will eat the lot.

Kidder and Manners, 1978

DEDICATION

TO MORLEY AND JANICE GABERT

ABSTRACT

Three experiments were carried out with early-weaned pigs, fitted with a simple T-cannula at the distal ileum, to determine the effects of supplementing diets with organic acids, oligosaccharides or lactitol on amino acid (AA) digestibility, microbial activity and bacterial populations in digesta collected from the distal ileum.

The effect of supplementing cornstarch-fish meal diets with 1% formic acid and increasing the level of Ca^{2+} and P was investigated in the first experiment. There was no effect ($P > .15$) of supplementing diets with formic acid or increasing the level of Ca^{2+} and P on the apparent ileal digestibilities of AA. Furthermore, there was no effect ($P > .08$) of supplementing diets with formic acid or increasing the level of Ca^{2+} and P on the pH, ammonia and volatile fatty acid (VFA) concentrations and bacterial populations in ileal digesta.

The effect of supplementing wheat-soybean meal diets with 1.5 and 3% fumaric acid (FA) or 1.5% sodium fumarate (NaFA) was investigated in the second experiment. Supplementation with 3% FA decreased ($P < .05$) the apparent ileal digestibilities of gross energy (GE), crude protein (CP) and some of the AA. Supplementation of diets with 1.5% FA or NaFA did not affect ($P > .05$) the apparent digestibilities of GE, CP or AA. However, the digestibilities of GE, CP and some of the AA exhibited linear ($P < .01$) decreases as FA level was increased. Supplementation of diets with FA or NaFA did not affect ($P > .12$) the pH or the concentrations of acetate and

propionate in ileal digesta. However, supplementation of diets with 1.5 and 3% FA increased (P < .05) the concentration of n-butyrate in ileal digesta.

The effect of supplementing barley-wheat-soybean meal diets with .2% galactooligosaccharides, .2% glucooligosaccharides or 1% lactitol was investigated in the third experiment. Supplementation with oligosaccharides or lactitol had little effect on the apparent ileal digestiblities of AA and monosaccharides. Except for galactose, supplementation of diets with oligosaccharides or lactitol did not affect (P > .13) the monosaccharide content or composition of ileal digesta. Furthermore, the output of monosaccharides, pH, ammonia and VFA concentrations and the bacterial populations in ileal digesta were not affected (P > .2) by supplementation with oligosaccharides or lactitol.

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LIST OF ABBREVIATIONS

NOT DEFINED IN THE TEXT

Abbreviation	**Definition**
ADG	Average Daily Gain
BW	Body Weight
CP	Crude Protein
DE	Digestible Energy
DM	Dry Matter
DMI	Dry Matter Intake
GE	Gross Energy
GLM	General Linear Model
HPLC	High Performance (Pressure) Liquid Chromatography
IU	International Unit
LSD	Least Significant Difference
ME	Metabolizable Energy
MJ	Megajoule
OM	Organic Matter
PBS	Phosphate-Buffered Saline
VFA	Volatile Fatty Acid(s)

CHAPTER 1

INTRODUCTION: THE EFFECTS OF SUPPLEMENTING DIETS WITH ORGANIC ACIDS, OLIGOSACCHARIDES AND LACTITOL IN EARLY-WEANED PIGS

The early-weaned pig, weaned at 3 to 4 wk of age, often exhibits low or no weight gain, low feed intake and often diarrhea. The period after weaning is often referred to as the "post-weaning check or lag period" and may persist for up to 2 wk. As was reviewed by Kidder and Manners (1978), the secretion of gastric HCl is relatively low during the first 3 to 4 wk of life and reaches a plateau by 8 to 10 wk. The secretions of pancreatic lipase, amylase and trypsin follow the same pattern (Kidder and Manners, 1978). Changes in intestinal histology and secretion following weaning are greatly influenced by diet (Miller et al., 1984; Hampson, 1986b). Within one day of weaning, villus height decreases resulting in a decrease in the small intestinal absorptive area emphasizing the need for a highly digestible diet (Hampson, 1986a). Supplementing diets with antibiotics to prevent diarrhea after weaning may result in selection of resistant strains of pathogenic bacteria. Therefore interest in "natural" feed additives has developed. "Natural" feed additives, such as organic acids, oligosaccharides and lactitol can also alter the intestinal microflora and may reduce the incidence of diarrhea.

Supplementation of diets for early-weaned pigs with organic acids (i.e. acetic, citric, formic, fumaric, lactic and propionic acid and the salts of these acids) has been investigated for many years (e.g. Cole et al., 1968; Kirchgessner and Roth-Maier, 1975). The supplementation of diets with organic acids increases ADG (Henry et al., 1985; Radecki et al., 1988; Giesting et al., 1991) and gain to feed ratio (G/F) (Falkowski and Aherne, 1984; Giesting and Easter 1985; Burnell et al., 1988). However, organic acid supplementation may not always have a significant effect on feed intake (FI), ADG or G/F (Henry et al., 1985; Bolduan et al., 1988; Risley et al., 1992). Many experiments have been carried out to investigate the effect of organic acid supplementation on animal performance but there is a lack of information on the mode of action of organic acids. Kirchgessner and Roth (1988) concluded that the effect may be due to: increased nutrient digestibility, decreased pH in the diet (and likely gastric pH) and decreased bacterial growth and improved pepsin activity, decreased intestinal bacterial growth and metabolic effects, especially with respect to energy. The response to organic acid supplementation depends on the type of diet (Burnell et al., 1988), level of acid (Giesting and Easter, 1985), type of acid (Radecki et al., 1988; Edmonds et al., 1985) and duration of feeding period (Kirchgessner and Roth, 1987b; Kirchgessner and Roth, 1990). Excellent reviews, including those by Kirchgessner and Roth (1982; 1988), Easter (1988) and Ravindran and Kornegay (1993) have been presented previously. The effects of each organic acid on animal performance, nutrient and energy retention and digestibility, compounds in the gastrointestinal tract (GIT) and microflora and metabolism will be summarized in this review.

Compared to organic acids, information on the supplementation of early-weaned pig diets with oligosaccharides or lactitol is limited. Like organic acids, oligosaccharides and lactitol can modify the intestinal microflora. They can promote the growth of lactic acid bacteria, e.g. Bifidobacterium and Lactobacillus, which suppresses the growth of pathogenic bacteria, and reduces the formation of putrefactive products and ammonia in the intestine (van Velthuijsen, 1979; Modler et al., 1990; Nousiainen and Setala, 1990).

A. Organic Acids

1. Acetic Acid

Acetic acid is a liquid organic acid, with the chemical formula CH_3COOH and molecular weight 60.05. Acetic acid has a boiling point of 118 °C, is miscible with water and pK_a[1] = 4.74 (Merck, 1983).

a) Animal Performance

Supplementation of early-weaned pig diets with acetic acid has usually not resulted in improved performance. Zhang et al. (1986) found no effect of supplementing diets with sodium diacetate on FI, ADG or G/F. Roth and Kirchgessner (1988) also found no effect on FI, ADG or G/F when diets were supplemented with acetic acid. However, they reported that supplementation decreased the incidence of diarrhea.

---

[1] $pK_a = -\log K_a$; where K_a is the acidity constant.

b) Metabolic Effects

Acetate has been shown to have a N-sparing effect. Imoto and Namioka (1983) fed growing pigs acetate, in the form of triacetin, at a rate of 5 and 10% of ME intake. Weight gain and N-retention showed a linear relationship with increasing levels of acetate. The effect on weight gain was .09 g of gain/g of acetate and the average N-sparing effect was 32.9 mg N/g of acetate. A linear increase in RNA, protein content and organ weight was observed in the liver, heart and femoral muscle. However, the total DNA content did not change in these tissues, indicating that acetate had no effect on the number of nuclei in the cells.

2. Citric Acid

Citric acid (CA) is a crystalline organic acid, with the empirical formula $C_6H_8O_7$ and molecular weight 192.12. Anhydrous CA has a melting point of 153 °C and is relatively soluble in water, 59.2% wt/wt at 20 °C. Citric acid is a tricarboxylic acid; at 25 °C $pK_{a1} = 3.13$, $pK_{a2} = 4.76$ and $pK_{a3} = 6.40$ (Merck, 1983).

a) Animal Performance

Many studies have demonstrated improved performance as a result of supplementation with CA. However, an improvement has not always been shown. Supplementation of CA increased ADG (Edmonds et al., 1985; Henry et al., 1985) and G/F (Falkowski and Aherne, 1984; Giesting and Easter, 1985). However, several studies have shown that CA supplementation did not improve FI (Henry et al., 1985; Risley et al., 1991), ADG (Falkowski and Aherne, 1984; Radecki et al., 1988) or G/F (Edmonds et al., 1985; Risley et al., 1991). The levels of CA supplemented in these studies ranged from .75 to 3%.

b) Nutrient and Energy Retention and Digestibility

Falkowski and Aherne (1984) reported that supplementation with CA did not affect the apparent fecal digestibilities of CP and DM. Broz and Schulze (1987) reported similar results. However, the apparent fecal digestibilities of OM and GE were increased.

c) Compounds in the GIT and Microflora

Risley et al. (1991) reported that 1.5% CA supplementation had no effect on pH of the GIT contents, Cl^-, VFA, and non-VFA concentrations in the GIT. These parameters were measured at the end of a 5 wk trial after which the animals were slaughtered (19.1 kg). Similar results were reported by Risley et al. (1992). This raises the question of whether or not there would have been significant effects if the animals were slaughtered at different times, for instance at the end of each week. There may not be any beneficial effect of supplementing diets with organic acids 5 wk after weaning. By this time the digestive system of the early-weaned piglet is likely well developed. In addition, CA supplementation did not affect the number of total anaerobes, Lactobacillus, Clostridia, or Escherichia coli in the GIT.

d) Metabolic Effects

Citric acid supplementation has usually not affected the concentrations of blood metabolites. Supplementation with CA did not affect the concentrations of plasma inorganic P, Fe^{2+}, glucose, urea, total lipid, blood hemoglobin as well as Fe-binding capacity and transferrin saturation (Falkowski and Aherne, 1984; Grassman and Klasna, 1986; Broz and Schulze, 1987). However, Broz and Schulze (1987) observed a decrease in plasma Ca^{2+} in response to CA supplementation.

Citrate uptake from the intestinal lumen has been investigated using brush border membrane vesicles (BBMV) prepared from the proximal jejunum of calves (Wolffram et al., 1990) and pigs (Wolffram et al., 1992). The uptake of citrate and fumarate by BBMV occurs by a common Na^+-gradient dependent mechanism that appears to be specific for tri- and dicarboxylates (Wolffram et al., 1990, 1992). This investigation has implications for the elucidation of the modes of action of citric acid. The contribution of citric acid to the formation of ATP and possibly a subsequent N-sparing effect on glucogenic amino acids, such as glutamine, could facilitate the rapid developmental changes that are taking place in the digestive tract.

The effects of supplementation of rat diets with CA on intermediary metabolism in the liver have been investigated by Grassman and Kirchgessner (1979) and Grassman and Klasna (1986). There was no effect of CA supplementation on the activities of enzymes in the citric acid cycle. However, succinate dehydrogenase activity was increased in one study and decreased in another. Grassman and Kirchgessner (1979) reported that CA supplementation did not affect the activities of liver transaminases. However, Grassman and Klasna (1986) reported that CA supplementation increased liver glutamate dehydrogenase, glutamate oxaloacetate transaminase, and glutamate pyruvate transaminase activities.

3. Formic Acid

Formic acid (FoA) is a liquid organic acid, with the chemical formula HCOOH and molecular weight 46.02. Formic acid has a boiling point of 100.5 °C, is miscible with water and $pK_a = 3.75$ (Merck, 1983; Solomons, 1984).

a) Animal Performance

Many studies have demonstrated improved performance as a result of supplementation with FoA, calcium formate (CaFoA) or sodium formate (NaFoA). However, an improvement has not always been shown. Supplementation of FoA, CaFoA or NaFoA increased FI (Kirchgessner and Roth, 1987b; Kirchgessner and Roth, 1990), ADG (Bolduan et al., 1988; Kirchgessner and Roth, 1990) and G/F (Kirchgessner and Roth, 1987a; Kirchgessner and Roth, 1987b). However, several studies have shown that FoA, CaFoA or NaFoA supplementation did not improve FI (Kirchgessner and

Roth, 1987a; Bolduan et al., 1988), ADG (Kirchgessner and Roth 1987a; Pallauf and Hüter, 1993) or G/F (Kirchgessner and Roth, 1990; Kirchgessner and Roth, 1987b). The levels of FoA, CaFoA or NaFoA supplemented in these studies ranged from .35 to 2.7%.

Supplementation of diets with CaFoA has been shown to reduce the incidence of diarrhea (Kirchgessner and Roth, 1987a; Kirchgessner and Roth, 1990; Pallauf and Hüter, 1993).

Carcass measurements were not affected by CaFoA supplementation (Kirchgessner and Roth, 1989).

b) Nutrient and Energy Retention and Digestibility

Bolduan et al. (1988) reported no effect of FoA supplementation on N-retention, and OM and CP (both ileal and fecal) digestibilities. However, Eckel et al. (1992) reported that FoA supplementation increased the apparent fecal digestibility of CP and GE. Similar results were observed by Eidelsburger et al. (1992c). Pallauf and Hüter (1993) reported that supplementation of diets with CaFoA increased the apparent fecal digestibilities of OM, ash, crude fiber, and N-free extract. However, CaFoA supplementation did not affect N-retention but increased the apparent absorption of Ca^{2+} and Ca^{2+}-retention.

c) Compounds in the GIT and Microflora

Formic acid supplementation decreased the pH of stomach contents (3.8 vs 4.0) (Bolduan et al., 1988). No _E_. _coli_ were measured in the stomach of pigs fed diets supplemented with

FoA. Histological examinations of the stomach did not reveal any ulceration (Bolduan et al., 1988). The level of lactic acid in the stomach was decreased by FoA supplementation. However, there was no effect of FoA supplementation on total VFA or ammonia concentrations in the stomach or on lactic acid or ammonia concentrations or pH in the small intestine (Bolduan et al., 1988). Roth et al. (1992) reported that supplementation of diets with FoA did not affect the VFA concentrations in the stomach or small intestine and the composition of the microflora in the ileum, cecum, and colon. However, supplementation with FoA decreased the number of bacteria in the duodenum and jejunum.

d) Metabolic Effects

Bolduan et al. (1988) reported no effect of FoA supplementation on pH in the urine.

4. Fumaric Acid

Fumaric acid (FA) is a crystalline organic acid, with the empirical formula $C_4H_4O_4$ and molecular weight 116.07. Fumaric acid sublimes at 200 °C and is sparingly soluble in water, .63 g in 100 g of water at 25 °C. Fumaric acid is a dicarboxylic acid; at 25 °C $pK_{a1} = 3.03$ and $pK_{a2} = 4.54$ (Merck, 1983).

a) Animal Performance

Many studies have demonstrated improved performance as a result of supplementation with FA. However, an improvement has not always been shown. Supplementation of FA increased G/F (Falkowski and Aherne, 1984; Giesting and Easter, 1985;

Kirchgessner and Roth, 1987b). However, several studies have shown that FA supplementation did not improve FI (Falkowski and Aherne, 1984; Henry et al., 1985; Kirchgessner and Roth, 1987a), ADG (Falkowski and Aherne, 1984; Edmonds et al., 1985; Henry et al., 1985) or G/F (Kirchgessner and Roth, 1987a; Roth and Kirchgessner, 1989). The levels of FA supplemented in these studies ranged from .9 to 2%.

Giesting and Easter (1985) investigated the relationship between the level of FA supplementation and growth response. The addition of graded levels of FA (1, 2, 3, and 4%) resulted in linear increases in ADG and G/F. The optimum level of supplementation appeared to be 3% FA. Supplementation with 4% FA did not increase ADG or G/F. There was no interaction between the level or source of protein in the diet and the level of supplementation of FA (Giesting and Easter, 1985; Giesting et al., 1991). Giesting and Easter (1985) reported that supplementation of diets for finishing pigs with FA had no effect on animal performance.

Giesting et al. (1991) reported an interaction between FA and sodium bicarbonate supplementation for ADG and G/F. Average daily gain and G/F of pigs fed diets supplemented with both FA and sodium bicarbonate were higher than for diets supplemented with or without FA or sodium bicarbonate. The positive effects of sodium bicarbonate may have been due to the prevention of metabolic acidosis. Giesting et al. (1991) added antibiotics to their experimental diets which would have likely eliminated any effects of FA on the microflora.

Like CaFoA, FA supplementation can reduce the incidence of diarrhea (Kirchgessner and Roth, 1987a).

b) Nutrient and Energy Retention and Digestibility

Giesting and Easter (1991) reported that supplementation with FA did not affect the apparent ileal digestibilities of DM and N. Falkowski and Aherne (1984) and Eidelsburger et al. (1992a) observed that the apparent fecal digestibilities of CP and DM were not affected by FA supplementation. However, other studies have shown that FA supplementation increased the apparent fecal digestibilities of OM, ether extract, crude fiber, CP, and N-free extract (Kirchgessner and Roth, 1980; Kirchgessner et al., 1982; Pallauf et al., 1987). Radecki et al. (1988) reported that supplementation of FA did not affect DE, ME, N corrected ME, N balance, percent N retained, apparent fecal N digestibility, Ca^{2+} balance, percent Ca^{2+} retained, and P balance and retention. However, Kirchgessner and Roth (1980) reported that FA supplementation increased the apparent fecal digestibilities of CP, Ca^{2+}, and P as well as Ca^{2+} and P retention.

c) Compounds in the GIT and Microflora

Risley et al. (1991), reported that FA supplementation increased the concentration of fumarate in the stomach (444.2 vs 2.5 meq/dL) and jejunum (93.6 vs 0.0 meq/dL). However, supplementation with FA had no effect on the pH of the GIT contents, Cl^-, VFA, and non-VFA concentrations. Similar results were observed by Risley et al. (1992). In addition, there was no effect of FA supplementation on the number of total anaerobes, Lactobacillus, Clostridia, or E. coli in the GIT. However, Sutton et al. (1991) reported that supplementation with FA decreased the number of E. coli in the stomach (3.87 vs 4.46 $\log_{10}$/gram) and increased acetate (68.3 vs 52.3 mM), propionate (60.9 vs 36.3 mM) and total VFA (166.3 vs 123.6 mM) concentrations in the cecum. Gedek et al. (1992) observed a decrease in the numbers of several groups of enteric bacteria in the duodenum, jejunum, and ileum when diets were supplemented with FA.

d) Metabolic Effects

Fumaric acid supplementation does not affect the concentrations of blood metabolites. In a study with rats, Grassman and Klasna (1986) found that FA supplementation did not affect blood glucose, total lipid or urea concentrations. Eidelsburger et al. (1992b) reported no effect of FA supplementation on acid-base status. Falkowski and Aherne (1984) reported no effect of FA supplementation on plasma urea concentration.

As described under citrate, fumarate and citrate uptake from the intestinal lumen occurs by a common Na^+-gradient dependent mechanism that appears to be specific for tri- and dicarboxylates (Wolffram et al., 1990, 1992).

The effects of supplementation of rat diets with CA on intermediary metabolism in the liver have been investigated by

Tschiershwitz et al. (1982) and Grassman and Klasna (1986). There was no effect of FA supplementation on the activities of enzymes in the citric acid cycle. However, Tschiershwitz et al. (1982) reported that FA supplementation increased succinate dehydrogenase activity. Grassman and Klasna (1986) reported that fumarase activity in the cytosolic fraction was increased by FA supplementation. Fumaric acid supplementation increased liver transaminase (glutamate dehydrogenase, glutamate oxaloacetate transaminase, and glutamate pyruvate transaminase) activities.

5. Lactic Acid

Lactic acid (DL- or racemic) is a liquid organic acid (melting point of crystals = 16.8 °C), with the empirical formula $C_3H_6O_3$ and molecular weight 90.08. Lactic acid has a boiling point of 122 °C, is soluble in water and at 25 °C pK_a = 3.86 (Merck, 1983).

Thomlinson and Lawrence (1981), using piglets fitted with gastric cannulas, found that the addition of 1% lactic acid to drinking water decreased gastric pH. Lactic acid addition delayed the proliferation of E. coli 0141:k85(B), a strain responsible for E. coli gastroenteritis, and reduced piglet mortality. Bourdeau et al. (1991) reported that supplementation of lactic acid and glycerol to growing pig diets had no effect on FI, ADG, G/F, carcass composition, and meat quality.

6. Propionic Acid

Propionic acid is a liquid organic acid, with the empirical formula $C_3H_6O_2$ and molecular weight 74.08. Propionic acid has a boiling point of 141 °C, is miscible with water and at 25 °C $pK_a = 4.87$ (Merck, 1983).

a) Animal Performance

Many studies have demonstrated improved performance as a result of supplementation with propionic acid (PA) in the form of Luprosil-NC®[2], CaPA or NaPA. However, an improvement has not always been shown. Supplementation of PA increased ADG (Mathew et al., 1991) and G/F (Giesting and Easter, 1985). However, several studies have shown that PA supplementation did not improve FI (Bolduan et al., 1988; Giesting and Easter, 1985), ADG (Bolduan et al., 1988; Sutton et al., 1991) and G/F (Roth and Kirchgessner, 1982). The levels of PA supplemented in these studies ranged from .25 to 2%.

b) Nutrient and Energy Retention and Digestibility

Roth and Kirchgessner (1982) reported that supplementation with CaPA had no effect on N-balance and apparent fecal digestibilities of DM, GE or N. In a second study, supplementation with Luprosil-NC® increased the apparent fecal digestibilities of DM and GE. Bolduan et al. (1988) did not observe an effect of Luprosil-NC® supplementation on the apparent ileal and fecal digestibilities of OM and CP.

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[2] Luprosil-NC® contains propionic acid, 53.5%; ammonium hydroxide, 9.5%; 1,2-propane diol, 11.5%; water, 25.5% and is a product of BASF Corp., Chemicals Division, Parsippany, N.J.

Mosenthin et al. (1992) found similar results; 2% PA supplementation did not affect the apparent ileal digestibilities of DM, OM, CP, ash or GE. However, supplementation increased the apparent ileal digestibilities of some of the amino acids. Apparent fecal digestibilities were not affected.

c) Compounds in the GIT and Microflora

Propionic acid may or may not affect the microflora of the GIT. Bolduan et al. (1988) did not detect any E. coli in the stomach of pigs fed 1% Luprosil-NC®; however, when diets were supplemented with .3% PA E. coli were present. On the other hand, Sutton et al. (1991) did not observe an effect of supplementation with Luprosil-NC® or NaPA on Lactobacillus or E. coli in the GIT or VFA concentrations in the stomach, duodenum and cecum. However, supplementation with Luprosil-NC® decreased the acetate and total VFA concentrations in the colon. Similar results were found by Mathew et al. (1991). However, pigs fed diets supplemented with Luprosil-NC® had a higher number of Lactobacilli in the stomach and duodenum.

Mosenthin et al. (1992) reported that supplementation with PA decreased the concentration of cadaverine in cecal digesta. However, Porter and Kenworth (1969) concluded that the overall production of diamines, primarily cadaverine and putrescine, is not an indication of predisposition to diarrhea. They reported that the microflora of normal and scouring animals may produce similar levels of cadaverine and putrescine.

Histological examinations of the stomach did not reveal any evidence of ulceration due to supplementation with PA (Bolduan et al., 1988).

d) Metabolic Effects

Growing pigs fed diets supplemented with 9% PA had less backfat (Thacker and Bowland, 1980). This may have been due to a reduction in FI and(or) accumulation of methylmalonyl-CoA due to insufficient levels of vitamin B_{12} (a coenzyme for methylmalonyl-CoA mutase) for the conversion of L-methylmalonyl-CoA to succinyl-CoA (Thacker and Bowland, 1980). The activities of acetyl-CoA carboxylase and fatty acid synthase are greatly inhibited by methylmalonyl-CoA (Frenkel et al., 1973). Thacker and Bowland (1980) reported that supplementation with PA increased the amount of odd chain fatty acids (e.g., 15:0, 17:0 and 17:1) in backfat.

Thacker and Bowland (1981a) reported that supplementation with 6 and 9% PA decreased the concentration of total serum cholesterol. This may have been due to the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity by PA (Chen et al., 1984), the rate-limiting enzyme in cholesterol biosynthesis (Rodwell et al., 1976) and(or) reduced feed (energy) intake (Grundy, 1978). Similar results were found by Thacker et al. (1981b) and Thacker et al. (1982). However, recent work has shown that infusion of propionate (36 mmol/kg$^{.75}$) into the cecum of growing pigs increased total serum cholesterol and LDL (low density lipoprotein) cholesterol concentrations (Beaulieu and McBurney, 1992).

B. Oligosaccharides

a) Animal Performance

Supplementation of diets for early-weaned pigs with .25 or .5% Neosugar[3] increased ADG and reduced the incidence of diarrhea (Modler et al., 1990). Neosugar is a mixture of fructooligosaccharides, with $\beta(2\rightarrow1)$ linkages, consisting of approximately 28% 1-kestose (GF_2), 60% nystose (GF_3), and 12% 1^F-β-fructofuranosyl nystose (GF_4) (Tokunaga et al., 1989). Increased FI and ADG were also reported when Bifidobacterium globosum A was administered; however the incidence of diarrhea was not affected (Apgar et al., 1993). On the other hand, Farnworth et al. (1992) reported that supplementation with 1.5% Jerusalem artichoke tuber flour or Neosugar did not affect FI, ADG, G/F or the incidence of diarrhea. Jerusalem artichoke (Helianthus tuberosus) tubers have a relatively high content of fructooligosaccharides (Graham and Åman, 1986; Roberfroid et al., 1993). Supplementation of diets with 5 or 10% inulin, a fructooligosaccharide with more than 30 monomers joined by $\beta(2\rightarrow1)$ linkages, did not affect FI or weight gain of rats (Modler et al., 1990; Levrat et al., 1991).

Howard et al. (1993) reported that supplementation of fructooligosaccharide (FOS[4]) to liquid diets fed to neonatal pigs increased epithelial cell proliferation in the cecum and colon. Supplementation of rat diets with 20% O-β-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-D-glucose (4′-GL) decreased weight gain; the supplementation of 10% had no effect. Both 10 and 20% supplementation increased cecal weight (Hayashi et al., 1991).

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[3] Meiji Seika Kaisha Ltd., Saitama, Japan.

b) Nutrient and Energy Retention and Digestibility

Mammals do not produce β-fructosidases, therefore fructooligosaccharides, such as Neosugar, can be classified under dietary fiber (Trowell et al., 1976; Hidaka et al., 1986). This is also the case for other nondigestible oligosaccharides which are resistant to digestion (Roberfroid et al., 1993). The apparent duodenal and ileal digestibilities of fructooligosaccharides in Jerusalem artichoke tubers in growing pigs were 48 and 51%, respectively. The relatively high digestibility in the duodenum may have been due to acid hydrolysis and(or) tuber or bacterial enzyme hydrolysis in the stomach (Graham and Åman, 1986).

Supplementation of rat diets with inulin increased the absorption of Ca^{2+} and Mg^{2+} (Levrat et al., 1991). Hayashi et al. (1991) fed diets supplemented with 10 or 20% 4′-GL and observed increased fecal Na^{+} and K^{+} excretions, increased fecal wet weight and decreased urinary volume in rats.

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[4] Coors Biotech Inc., Westminster, CO.

c) Compounds in the GIT and Microflora

Fructooligosaccharides and other nondigestible oligosaccharides, such as transgalactosylated oligosaccharide, are readily fermented and act as selective growth substances for Bifidobacterium; so-called "bifidus-growth factors" (Tanaka et al., 1983; Faisant et al., 1991; Roberfroid et al., 1993). The benefits of this fermentation include a reduction in pH in the large intestine through the production of acetic and lactic acid which suppresses the growth of pathogenic and putrefactive bacteria (Hidaka et al, 1986; Modler et al., 1990; Roberfroid et al., 1993). In addition, E. coli and several species of Clostridium do not utilize fructooligosaccharides (Hidaka et al., 1986; Modler et al., 1990). Several other synthetic oligosaccharides (galsucrose, isogalactobiose and lactosucrose) did not support the growth of several strains of E. coli but supported the growth of Bifidobacterium in vitro (Minami et al., 1983). When humans consumed 8 g of Neosugar/d, Hidaka et al. (1986) observed a negative correlation between the number of Bifidobacterium and C. perfringens suggesting that the growth of C. perfringens is being inhibited by the production of acetic and lactic acid. Bifidobacterium can produce the following enzymes to hydrolyse oligosaccharides: α- and β-galactosidase and α- and β-glucosidase (Desjardins and Roy, 1990).

Supplementation of diets with 10% inulin increased VFA concentration in the cecum, especially propionate, in rats

(Levrat et al., 1991). Supplementation of rat diets with 10% 4'-GL stimulated the growth of Bifidobacterium in the cecum (Ohtsuka et al., 1990). On the other hand, Farnworth et al. (1992) reported that supplementation of diets for early-weaned pigs with Jerusalem artichoke tuber flour or Neosugar did not affect VFA concentrations or the number of bacteria in the colon. Similar results were reported by Howard et al. (1993) when liquid diets for neonatal pigs were supplemented with FOS.

e) Metabolic Effects

The metabolism of Neosugar was investigated by Tokunaga et al. (1989). Following oral administration of [U-^{14}C]Neosugar to rats, approximately 55% of the radioactivity being expired as $^{14}CO_2$ whereas 3.2% and 3% were excreted in urine and feces within 24 h, respectively. To illustrate that Neosugar is rapidly fermented by intestinal bacteria [U-^{14}C]Neosugar was anaerobically incubated with rat cecal contents. After 6 h, 12% of the radioactivity was collected as $^{14}CO_2$, 11% in microbes, and 72% in VFA. Neosugar is exclusively metabolized by intestinal bacteria, Oku et al. (1984) reported that intravenously injected Neosugar was excreted in the urine and not metabolized by endogenous enzymes.

C. Lactitol

a) Animal Performance

Calves, like early-weaned pigs, often suffer from diarrhea. The supplementation of milk replacers with lactitol

(4-O-β-D-galactopyranosyl-D-sorbitol), 1 to 4 g/kg metabolic live weight ($W^{.75}$) per day, promoted the growth of calves (Nousiainen and Setala, 1990). However, Ammann, et al. (1988) reported that supplementation of diets with 2.5 g lactitol/kg BW per day did not affect FI or weight gain in rats.

b) Nutrient and Energy Retention and Digestibility

Lactitol is not absorbed in the human small intestine, and is not hydrolysed to any significant extent by mammalian enzymes (Patil et al., 1987). This was confirmed by Harju (1988a,b) using mucosal homogenates from the duodenum of calves, humans and pigs. Ahrens and Schön (1988) fed wheat-soybean meal diets, supplemented with 5 or 7.5% lactitol, to miniature pigs and observed a trend for increased bacterial N-excretion (1.2 vs .9 g/d) and decreased apparent fecal N digestibility (88.9 vs 90.7). However, the bacterial-N corrected apparent fecal digestibilities were similar (95.3 vs 94.8). The supplementation of diets with 2.5 g lactitol/kg BW per day decreased the fecal excretion of Ca^{2+} and increased absorption and retention of Ca^{2+} in rats. However, excretion, absorption, and retention of P were not affected by supplementation with lactitol. The increased intestinal absorption of Ca^{2+} may have been due, in part, to an increased availability of Ca^{2+} in the large intestine, which could be associated with a decrease in pH. The pH of digesta in the cecum and of feces was lower for rats fed diets supplemented with lactitol, 7.1 vs 7.4 in the cecum and 7.3 vs 7.9 in the feces, respectively (Ammann et al., 1986; Ammann et al., 1988).

c) Compounds in the GIT and Microflora

Lactitol is metabolised by bacteria in the colon (Saijonmaa et al., 1978). Bird et al. (1990) have shown that incorporation of lactitol into the diets of miniature pigs can produce a 20% increase in fecal N excretion. Studies carried out by van Velthuijsen (1979) have shown that E. coli do not ferment lactitol to acids or gasses. However, several Lactobacillus sp. produce a large amount of acids, the same amount from lactitol as from lactose. The predominant acid formed from the fermentation of lactitol by Lactobacillus is L-(+)-lactic acid. Bifidobacteria produce less acids from the fermentation of lactitol than from lactose.

When lactitol was used as a substrate, the relative activities of β-galactosidase (lactase) in Bifidobacterium longum and Lactobacillus acidophilus were 95 and 96 while the activities of typical proteolytic bacteria, Bacteroides fragilis and E. coli, were 8 and 2, respectively. This difference in enzyme specificity gives the lactic acid bacteria a growth advantage, especially in the large intestine where numbers are higher than in the small intestine. The lactic acid bacteria ferment galactose and sorbitol to acids which decreases the pH in the intestine and slows the growth of proteolytic bacteria. The intestinal flora then becomes lactic acid predominant (Nousiainen and Setala, 1990; Kitler et al., 1992). Ahrens and Schön (1988) reported that supplementation with 5 or 7.5% lactitol decreased pH, ammonia concentration and acetate to propionate ratio in the cecum 8 h postprandial in miniature pigs.

Lactitol has been used in the treatment of chronic hepatic encephalopathy (Lanthier and Morgan, 1985). The beneficial effects of lactitol may be due to a reduction in the pH of the contents in the colon, therefore reducing the absorption of un-ionized ammonia, increasing the incorporation of ammonia into bacterial protein, and decreasing intestinal transit time (cathartic effect) (Lanthier and Morgan, 1985; Patil, et al. 1986). A lower pH in the colon would also reduce the activity of proteolytic bacteria therefore reducing ammonia production and serum ammonia concentration (Harju, 1988b).

D. Summary

The supplementation of diets with organic acids, oligosaccharides or lactitol can improve the performance of early-weaned pigs. However, an improvement is not always observed. Improved performance may be due to increased nutrient and energy retention and digestibility, alteration of bacteria in the GIT, bacterial metabolites and other compounds in the digestive tract or an effect on metabolism.

However, there is a scarcity of information on the influence of supplementation with organic acids on the digestibility of amino acids in diets for early-weaned pigs, especially when these are determined with the ileal analysis method (e.g., Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). There is a scarcity of information on the effects of organic acid supplementation on the small intestinal microflora shortly after weaning. There is also a scarcity of information on the effect of supplementation with oligosaccharides and lactitol on the digestibility of nutrients measured at the distal ileum as well as on populations and metabolism of bacteria.

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CHAPTER 2

THE EFFECT OF SUPPLEMENTATION OF FORMIC ACID, CALCIUM AND PHOSPHORUS ON THE DIGESTIBILITY OF AMINO ACIDS AND BACTERIAL POPULATIONS AND METABOLITES IN THE DIGESTIVE TRACT OF EARLY-WEANED PIGS

A. Introduction

Organic acid supplementation to diets for early-weaned pigs has been shown to alleviate the post-weaning depression in growth by improving ADG and feed efficiency (e.g., Giesting and Easter, 1985; Bolduan et al., 1988) and to reduce the incidence of diarrhea (Kirchgessner and Roth, 1987, 1990). As was reviewed by Kirchgessner and Roth (1988), the growth-promoting effects of different organic acids may be attributed to: 1) improvement in the digestibility of nutrients and energy; 2) gastrointestinal effects, including antimicrobial effects in which potentially detrimental bacteria are inhibited as a result of a reduction in the gastric pH; 3) changes in intermediary metabolism, including the efficiency of energy utilization.

The objectives of this study were to investigate the effects of supplementation of 1% formic acid to diets for early-weaned pigs with normal and high Ca^{2+} and P levels on the ileal and fecal digestibilities of GE, OM, CP, ash and amino acids (AA). An additional objective was to measure the concentrations of microbial metabolites (ammonia and VFA) and bacterial populations in digesta collected from the distal ileum. The high Ca^{2+} and P content of two of the diets served to increase the buffer capacity. This would allow for the assessment of the influence of buffer capacity on the aforementioned parameters and if supplementation of high mineral diets with formic acid could reverse or enhance these effects.

B. Materials and Methods

Animals and Diets

Twelve crossbred[1] barrows, weaned at 3 wk, initial BW 7.8 ± .6 kg, were obtained from a local pig farm. The pigs were housed in stainless steel metabolic crates in a temperature-controlled room (25 to 27 °C). An 18% CP non-medicated starter diet (Sauer et al., 1983) was fed for 1 wk before surgery. The pigs had ad libitum access to feed and water. A simple T-cannula was surgically inserted approximately 5 cm anterior to the ileo-cecal sphincter according to procedures adapted from Sauer et al. (1983) and Li et al. (1993) with the following modifications. Soft polyvinylchloride plastisol cannulas were prepared using the procedures outlined by Li et al. (1993). Anaesthesia was induced by i.m. injection of .3 mg atropine sulfate[2], 2.0 mg azaperone[3], followed by i.p. injection of 13.0 mg metomidathydrochloride[4]/kg BW. Electrolytes[5] were infused (9 to 15 mL/h) via an ear vein during and 2 to 6 h following surgery to possibly hasten recovery and maintain electrolyte balance. The cannula was fastened with two purse string sutures using 3-0 chromic absorbable suture. The cannula was exteriorized on the left side of the pig, parallel to the last two ribs. Before the incision was closed, 5 mL of Penicillin-G[6] was given i.p.. Lincomycin-Spectinomycin[7] (1 mL) was given i.m. for 3 to 5 d following surgery. Karaya paste[8] was liberally applied three times daily behind the retaining ring to minimize leakage for the duration of the experiment.

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[1] The pigs were products from a cross breeding system involving the following breeds: German Landrace, Duroc, Piétrain, Belgian Landrace and Hampshire. Hülsenberger Swine Breeding Program, H. Wilhelm Schaumann, Forschungszentrum für Tierernährung und Tierzucht, Versuchsgut Hülsenberg D-2362 Wahlstedt, FRG.

The pigs were immediately returned to the metabolic crates after surgery and fasted that same day. Starting the next day, 20 mL of electrolytes[5] were given at 0600, 1300, and 2100 and 20 mL of dextrose solution (10% wt/vol) at 1000 and 1600 orally with a 20 mL syringe. The next day 20 g of starter diet (Sauer et al., 1983) was fed at 0600, 1000, 1300, 1600 and 2100; this amount was increased each day until the pigs consumed the diet at a rate of 4% of BW per day. The pigs were allowed a 7 d recuperation period. During the experiment the pigs were fed at 0600, 1400 and 2200. Feed intake was determined for each pig after the pigs were weighed the day before the start of each experimental period; 348 ± 28 g/d for period one and 425 ± 38 g/d for period two. The average BW of the pigs at the beginning of the first and second experimental periods were 8.7 ± .7 kg and 10.6 ± 1.0, respectively. The weight of the pigs at the conclusion of the experiment was 13.8 ± 1.2 kg. The pigs were given ad libitum access to water before and after feeding. The pigs usually consumed their meal allowance within 20 min.

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[2] Atropinum sulfuricum solutum 1%; 10 mg atropine sulfate/mL; Wirtschaftsgenossenschaft Deutscher Tierärzte eG, Dreyerstraße 8-12 3000 Hannover 1, FRG.

[3] Stresnil™; 40 mg azaperone/mL; Janssen GmbH, 4040 Neuss 21, FRG.

[4] Hypnodil™; 50 mg metomidathydrochloride/mL; Janssen GmbH, 4040 Neuss 21, FRG.

[5] Strerofundin® G-5; B. Braun Melsungen AG, 3508 Melsungen, FRG. The following nutrients were provided per mL of infusate: 5.55 mg NaCl; .3 mg KCl; .2 mg $MgCl_2 \bullet 6H_2O$; .37 mg $CaCl_2 \bullet 2H_2O$; 5.05 mg Na-lactate; 55 mg glucose monohydrate.

[6] Procain-Penicillin-G; 300 000 I.E. benzylpenicillin-procain $1H_2O$/mL; Tad Pharmazeutisches Werk GmbH, D-2190, Heinz-Lohmann-Straße 5, Cuxhaven 1, FRG.

[7] Lincospectin®; 56.7 mg lincomycinhydrochlorid-monohydrate (50 mg Lincomycin) and 151.2 mg spectinomycinsulfate-tetrahydrate (100 mg spectinomycin)/mL; Upjohn GmbH, 6148 Heppenheim, FRG.

[8] Stominal® Anus-praeter-Hygiene Karaya-Paste No. 9539 Dansac A/S DK-3480, Frendesborg, Denmark.

Four semi-purified diets, based on cornstarch and fish meal, were formulated to contain 18% CP (N x 6.25). The formulation of the experimental diets is presented in Table 2-1. The diets were formulated to meet or exceed NRC (1988) standards. The diets with normal levels of Ca^{2+} and P and with or without 1% (wt/wt) formic are referred to as +NM or -NM, respectively. The diets with high levels (double the +NM and -NM diets) of Ca^{2+} and P and with or without 1% formic acid are referred to as +HM and -HM, respectively. The additional calcium carbonate and dicalcium phosphate were included at the expense of cornstarch to double the level of Ca^{2+} and P.

The fish meal[9] was of South-American origin. The fish meal was sieved through a 5-mm mesh screen before diet formulation to remove sharp scales and bones; samples for analyses were taken after sieving. Dextrose was included in the diets to possibly improve palatability. Cellulose was included to increase the fiber content of the diets and possibly decrease the incidence of constipation. Soybean oil was included to reduce dustiness, improve palatability and meet NRC (1988) standards for DE. Vitamins and minerals were supplemented to meet or exceed NRC (1988) standards. Chromic oxide (.5%) was included in the diet as marker for the determination of nutrient and energy digestibilities.

The experiment was carried out according to a two-period changeover design (Gill and Magee, 1976). Each experimental period comprised 11 d. Feces were collected on d 6 and 7. Ileal digesta were collected on d 8 and 9 for a total of 24 h according to procedures adapted from Li et al. (1993). Digesta were collected from 0600 to 1400, 2200 to 0600 and 1400 to 2200. Digesta were collected by the use of plastic tubing (width 3 cm; length 20 cm) heat-sealed at one end and fastened to the barrel of the cannula by a Velcro-elastic strap. Before the tubing was attached, digesta were removed from the barrel of the cannula with a spatula. Each time the collection bag was emptied, a subsample of digesta was taken, transferred to a polyethylene bottle and immediately frozen at -28°C. The remainder of the digesta were immediately frozen at -28°C. Digesta for bacterial counts were collected on d 11 from 0600 to 1400. During the collection the digesta were kept at 4°C in a sealed polyethylene bottle. At the end of the collection period digesta were mixed and an aliquot transferred to a 5.5 mL glass vial (there was no headspace) and capped. The vials were vacuum-sealed in a polyethylene bag with a CO_2-generating and O_2-binding anaerobic pack[10] and delivered to the Institute of Medical Microbiology, Ludwig-Maximilians University, Munich, Germany.

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[9] Obtained from Fritz Köster Handelsgesellschaft, GmbH & Co. 2000 Hamburg 13, FRG.

Chemical and Statistical Analysis

Digesta and feces were freeze-dried, pooled within pig and period, ground in a Wiley mill[11] through a .8-mm screen and mixed before analysis. The diets and fish meal were ground similarly. Analysis for DM, OM, CP, ether extract, Ca, P, and ash were carried out according to AOAC (1990). The pH of the diet was determined according to Risley et al. (1992). Twenty grams of each diet were weighed in triplicate into 250-mL beakers and 100 mL of distilled and deionized water were added. The slurry was mixed with a magnetic stirrer and the pH was measured[12]. Buffer capacities of the experimental diets were measured according to Srilaorkul et al. (1989). Ten grams of each diet in 50 mL of distilled and deionized water were titrated with .1 or 1 N HCl until pH 1 was reached. Similarly, 20 g of each diet in 100 mL of distilled and deionized water were titrated with .1 N NaOH until pH 10 was reached. The titrations were performed in duplicate until equilibrium (usually 4 min) and the slurry was continuously mixed with a magnetic stirrer. The buffer index was calculated using the following equation:

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[10] Anaerocult®; contains kieselgur, iron powder, citric acid and sodium carbonate. P, E. Merck, Postfach 41 91, D-6100 Darmstadt 1, FRG.

[11] Arther H. Thomas Co., Philadelphia, PA.

$$dB/dpH = \frac{(\text{mL acid or base})(\text{normality factor})}{(\text{volume of sample})(\Delta pH)}$$

Buffer capacity curves were obtained by plotting the buffer index versus pH. Gross Energy was determined using a Parr 1241 Adiabatic Oxygen Bomb Calorimeter[13]. Chromic oxide was determined according to the procedure of Fenton and Fenton (1979).

For AA analysis, approximately 100 mg of sample was weighed into a screw-capped test tube, mixed with 3 mL of 6 N HCl, flushed with N_2 and hydrolyzed at 110°C for 24 h. Analyses were performed in duplicate. The samples were mixed with the internal standard (DL-amino-n-butyric acid), centrifuged at 1,110 x g for 15 min and analyzed according to Jones and

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[12] Eydam, Kiel, FRG.

[13] Parr Instrument Company, Moline, IL.

Gilligan (1983) using a Varian[14] 5000 HPLC. A reverse-phase column was used following the procedures previously described by Dugan et al. (1989). Amino acids were derivatized with an o-phthaldialdehyde reagent solution and detected spectrofluorometrically. Methionine, cysteine, and tryptophan were not determined.

The concentrations of VFA in digesta were determined with a gas chromatograph[15] using the method of Kaufmann and Hagemeister (1969), modified according to Mosenthin (1987). Free ammonia in digesta was determined as described by Fawcett and Scott (1960). The pH of digesta was also measured[12]. Bacterial counts were carried out according to Gedek et al. (1992). One gram of digesta from each pig was diluted 1:10 (1 g in 9 mL) in sterile PBS (pH 7.2). Following 1 min of vigorous shaking, serial dilutions were made until the final dilutions were 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9}, respectively. From each tube .1 mL was transferred to a selective media and spread with a glass rod. Colonies were counted on plates that contained 20 - 200 colonies. The appropriate dilution factors and volume applied to each plate were used to calculate the initial number of bacteria per gram of digesta.

Analysis of variance was carried out according to Gill and Magee (1976) with pig as block and period and diet as main effects using the GLM procedure of SAS (1988). Interactions could not be tested because the pigs received a different diet in period two. Least squares means were employed due to the unbalanced design. Each pig was not fed each diet. Period and diet least squares means were compared using Fisher's LSD procedure (Milliken and Johnson 1984). This procedure does not allow for multiple comparisons unless a significant F-value is detected ($P < .05$). Log_{10} transformation of bacterial counts were performed before statistical analysis.

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[14] Varian Associates, Sunnyvale, CA.

[15] Model 8400, Perkin-Elmer Ltd., Buckinghamshire, England.

C. Results and Discussion

The pigs remained healthy and consumed their meal allowances throughout the experiment. Post-mortem examinations, carried out at the conclusion of the experiment, revealed no intestinal adhesions. The chemical composition of fish meal is presented in Table 2-2. The composition is comparable to fish meal from Chile used by Wiseman et al. (1991) and three different fish meals used by Knabe et al. (1989). Fish meal is high in CP and minerals, predominantly Ca^{2+} and P. Fish meal also has a relatively high content of indispensable AA.

The chemical composition of the diets is presented in Table 2-3. The concentrations of Ca^{2+} and P in the HM diets was approximately double that in the NM diets. The addition of 1% formic acid decreased the pH of the diets, 6.25 and 6.13 for the -NM and -HM diets, and 4.46 and 4.59 for the +NM and +HM diets, respectively. Bolduan et al. (1988) observed that the supplementation of .35% formic acid decreased ($P < .05$) the pH in the stomach (4.0 vs 3.8) and increased ($P < .05$) ADG (349 vs 275 g/d). The buffer capacity curves for the experimental diets are shown in Figure 2-1. The buffer capacities of the experimental diets were low when pH was greater than five. Between pH 5.5 and 8 the buffer capacities of the +NM and +HM diets were slightly higher than for the -NM and -HM diets; likely due to the presence of formic acid. For all of the diets, the buffer capacities increased steadily as pH decreased. When pH was decreased from 2 to 1 there were large increases in buffer capacities. The increases in buffer capacities with decreasing pH were likely due to the relatively high carbonate and phosphate contents of the diets. The solubility of phosphates increases under acidic conditions and ortho- and pyrophosphates have a high buffer capacities when pH is low (Molins, 1991). Calcium carbonate is sparingly soluble in water but is soluble in acids (CRC, 1980). The additional calcium carbonate and dicalcium phosphate added to the HM diets increased the buffer capacities between pH 1.5 and 4 compared to the NM diets. However, the addition of formic acid increased the buffer capacities of both the +NM and +HM diets between pH 2 and 4.5 compared to the -NM and -HM diets, respectively. The increases in buffer capacities after formic acid addition may have been due to increased carbonate and phosphate solubilities in the diets.

The growth-promoting effects of formic acid are likely due to a reduction in diet pH and subsequent reduction of pH in the stomach. Formate can be cleaved in the digestive tract to CO_2 and H_2 by formate-hydrogen lyase. Both Escherichia coli and Enterobacter aerogenes can produce this enzyme (Gottschalk, 1986). Methanogenic bacteria, primarily located in the large intestine, can also reduce CO_2 to CH_4 to synthesize ATP (Gottschalk, 1986; McCarthy and Salyers, 1988).

The apparent ileal and fecal digestibility coefficients of GE, DM, OM, CP, ash and AA of the experimental diets are presented in Tables 2-4 and 2-5, respectively.

The ileal digestibilities of ash of both HM diets were lower ($P < .01$) than those of both NM diets. This was likely due to the higher Ca^{2+} and P content of the HM diets. Absorption of Ca^{2+} and P appears to occur in the duodenum by both passive (diffusion) and active transport mechanisms (McDowell, 1992). The excess Ca^{2+} and P in the HM diets were likely not absorbed to a great extent. The ileal digestibilities of OM were decreased ($P < .05$) when diets were supplemented with formic acid, reguardless of mineral level. There were no differences ($P > .15$) in the apparent ileal digestibilities of CP, indispensable and dispensable AA between the diets. Giesting and Easter (1991) reported no effect ($P > .10$) of supplementation with 2% fumaric acid on the ileal digestibility of DM and N. Supplementation of diets with .35 or 1.3% formic acid did not affect ($P > .05$) the ileal digestibilities of OM and CP (Bolduan et al., 1988).

However, Mosenthin et al. (1992) observed an increase ($P < .04$) in the apparent ileal digestibilities of several AA when diets for growing pigs were supplemented with 2% propionic acid. The lack of response in the present studies may have been due to the relatively high digestibility of fish meal. The apparent digestibility coefficients for AA in this study were in the range of values reported by Wiseman et al. (1991) and Knabe et al. (1989). However, the digestibilities were higher than the values reported by Jørgensen et al. (1984). These differences may have been due to differences in AA content of the diets. The AA content of the assay diet can explain a large amount of the variation in apparent ileal digestibilities. This is due to the contribution of endogenous AA relative to dietary AA in ileal digesta (Fan et al., 1993). In addition, some of the differences in digestibilities may have been due to differences in processing methods. For instance, excessive heat treatment (160°C) reduces ($P < .05$) the ileal digestibility of AA (Wiseman et al., 1991).

For each diet, the apparent ileal digestibilities of arginine and lysine were relatively high. In contrast, the digestibilities of threonine and glycine were relatively low, which was also observed by Knabe et al. (1989). The relatively high apparent ileal digestibilities of arginine and lysine and low digestibility of threonine tend to support the hypothesis that specificity of enzymes is an important factor in determining apparent AA absorption in the small intestine. As was discussed by Low (1980), of the indispensable AA, arginine and lysine would be expected to appear first after enzymatic hydrolysis and threonine last based on the specificity of the proteases and peptidases involved in digestion. The relatively low apparent ileal digestibility of threonine may result, in part, from its relatively low rate of absorption. Using temporarily-isolated loops in growing-finishing pigs, Buraczewska (1979) investigated the ability of different segments of the small intestine to absorb peptides and AA. Of the indispensable AA, the absorption rates in segments of the middle and distal small intestine were highest for arginine, methionine, isoleucine and leucine and lowest for threonine and histidine. The relatively low apparent ileal digestibilities of threonine and glycine may also, in part, result from their relatively high concentrations in endogenous secretions. A relatively high level of glycine and threonine in digesta collected from the distal ileum of growing pigs fed a protein-free diet was observed by Holmes et al. (1974) and Sauer et al. (1977). Glycine accounts for more than 90% of the total AA secreted in porcine bile juice (Souffrant 1991) and is a constituent of bile salt conjugates. Approximately 95% of the conjugated bile salts are re-absorbed via active transport and enter the enterohepatic circulation in the distal ileum (Weiner and Lock, 1968). The conjugated bile salts which are not reabsorbed can be deconjugated by microbes (Macdonald et al., 1983). The deconjugated glycine escapes re-absorption and enters the large intestine (Shiau, 1987). Furthermore, the small intestinal secretions, which include mucins, supply the largest proportion of N to endogenous N in the small intestine (Auclair, 1986). "Native" mucin which represents over 95% of mucin glycoprotein is very rich in threonine, serine and proline (Neutra and Forstner, 1987).

The apparent fecal digestibility of GE was lower ($P < .01$) for the -HM than the -NM diet. However, the apparent fecal digestibilities of ash were lower ($P < .01$) for the HM than the NM diets. This was likely due to the higher Ca^{2+} and P content of the HM diets, as was previously discussed. Supplementation with formic acid increased ($P < .01$) the apparent fecal ash digestibility in the +NM diet. Eckel et al. (1992) reported that supplementation with .6, 1.2, 1.8, and 2.4% formic acid increased ($P < .05$) the apparent fecal digestibility of CP and GE. Similar results were reported by Eidelsburger et al. (1992).

The net disappearance of DM, OM, CP, ash and AA in the large intestine, expressed as grams per kilogram DMI, is presented in Table 2-6. The net disappearance of DM and OM for the +HM diet was higher ($P < .05$) than for the other diets. The net disappearance of CP and AA was highest for the +HM diet. This is likely a result of the lower ileal digestibility and a subsequently larger disappearance in the large intestine. There was net synthesis of lysine ($P < .02$) in pigs fed the -HM diet. Net synthesis was also observed for isoleucine and tyrosine. The net disappearance for CP and other AA was lower for the -HM than for the other diets. This indicates that there was a decrease in microbial activity in the large intestine. There was a trend (P < .14) for the fecal digestibility of isoleucine, lysine and threonine to be lower in the -HM than in the other diets.

For each diet, there was a larger disappearance of the dispensable than of the indispensable AA. Of the indispensable AA, the disappearance was usually highest for leucine and threonine. Of the dispensable AA, aspartic acid, glutamic acid and glycine disappeared to the largest extent. Proteins secreted in pancreatic and small intestinal juice contain relatively large amounts of aspartic acid, glutamic acid and leucine (Corring and Jung 1972; Buraczewska, 1979). The relatively large disappearance of these AA in the large intestine may indicate preferential fermentation of undigested endogenous protein (or unabsorbed AA or peptides) by the microflora in the large intestine.

The average apparent ileal as well as fecal digestibilities of GE, DM, OM, CP, ash and AA for each experimental period are presented in table 2-7. The ileal digestibilities of CP, arginine, isoleucine, leucine, phenylalanine, threonine, valine, alanine and glycine were higher (P < .05) in period two than in period one. For the indispensable AA, the differences ranged from 1.9 to 3.3 percentage units for arginine and threonine, respectively. The apparent fecal digestibility of GE and OM was higher (P < .02) in period two than in period one. The appparent fecal digestibility of ash was lower (P < .03) in period two than in period one. However, experimental period did not affect (P > .2) the apparent fecal digestibilities of AA. The aforementioned results support the premise that the ileal analysis method is more sensitive than the fecal analysis method for detecting differences in AA digestibilities (Sauer and Ozimek, 1986). The observed period differences in ileal digestibility are likely a manifestation of further development of the digestive tract as the animals increase in age. The activities of digestive enzymes, with the exception of rennin and lactase, which decline after 7 d, increase until the age of approximately 8 wk (Longland, 1991). Wilson and Leibholz (1981) reported higher digestibilities of DM (P < .05) and N (P > .05) in pigs of 19 to 24 d of age compared to pigs of 10 to 15 d of age.

The pH, ammonia and VFA concentrations in ileal digesta are presented in Table 2-8. There was no effect (P > .2) of diet on the pH of ileal digesta. The concentration of ammonia in digesta from pigs fed the +NM diet tended to be lower (P < .08) than for the pigs fed the other diets. This may indicate that bacterial activity was suppressed in the stomach and small intestine.

There was no effect (P > .2) of diet on the VFA concentration in ileal digesta. The VFA concentration in ileal digesta of pigs, (except for acetate, which was higher, 19.8 vs an average of 10.7 mM), was similar to that of growing pigs fed a low fiber semi-purified diet (Sauer et al., 1991). Sauer et al. (1991) used soybean meal as protein source; soybean meal contains non-starch polysaccharides which are fermented to VFA by the intestinal microflora. This likely explains the higher acetate content in ileal digesta. Other workers have reported that the supplementation of diets with organic acids does not affect (P > .10) the concentrations of VFA in the gastrointestinal tract (e.g., Risely et al., 1991, 1992; Roth et al., 1992).

However, there were significant (P < .03) effects of diet on the percentages (mol/100 mol) of VFA in ileal digesta. Acetate accounted for 75 to 80% of the VFA in ileal digesta. Digesta collected from pigs fed the -HM diet had a lower (P < .02) percentage of acetate, compared to the -NM and +NM diets, and a higher (P < .03) percentage of n-butyrate. The percentage of n-butyrate in digesta from pigs fed the -NM diet was lower (P < .03) than for pigs fed the other diets. Conversely, the percentage of n-butyrate in digesta from pigs fed the -HM diet was higher (P < .03) than for pigs fed the other diets.

The $\log_{10}$ of the number of bacterial cells per gram of fresh ileal digesta is presented in Table 2-9. Pigs fed the -NM and +HM diets had a lower (P < .11) number of enterococci. There was no effect (P > .11) of diet on the remaining populations of bacteria. Some of these populations are not mutually exclusive and some of the bacteria were assayed more than once. The most predominant group were the Gram-positive anaerobes, while the least predominant group were the enterococci. There was no effect (P > .15) of diet on the ileal output of bacteria (bacterial cells/kg DMI). However, the aforementioned results should be interpreted with caution due to the importance of bacteria attached to the mucosa of the stomach and small intestine, as reviewed by Conway (1989). The results of this study agree with those of Risley et al. (1992). Kirchgessner et al. (1992) also reported that supplementation with 1.25% formic acid did not change (P > .05) the composition of the microflora in the ileum, cecum and colon. However, the supplementation with 1.25% formic acid decreased (P < .05) numbers of all groups of bacteria in the duodenum and jejunum. Relatively large populations of lactobacilli were not established in the gastrointestinal tract by formic acid supplementation in this study. A relatively low pH favors the growth of lactobacilli. The beneficial effects of Lactobacillus include protection against pathogen colonization (Muralidhara et al., 1977), and decreased incidence of diarrhea (Lucky, 1984). However, when attempts are made to confirm that Lactobacillus improve the health of the host, nonsignificant results are often reported (Conway, 1989).

In summary, there was no effect (P > .15) of supplementing diets with formic acid or increasing the level of Ca^{2+} and P on the apparent ileal digestibilities of AA. There was no effect (P > .08) of supplementation with formic acid or increasing the level of Ca^{2+} and P on the pH, ammonia and VFA concentrations and bacterial populations in ileal digesta.

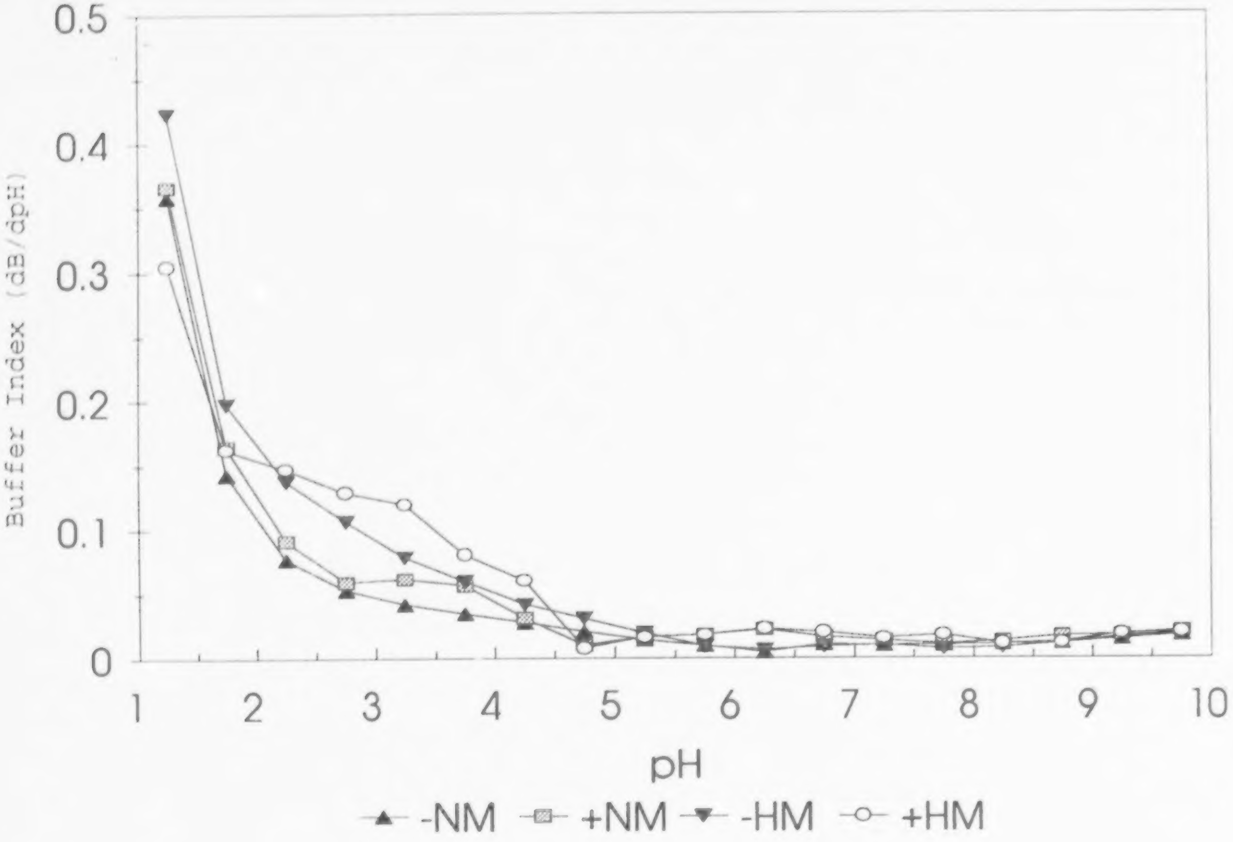

Figure 2-1. Buffer capacity curves for the experimental diets following titration with .1 or 1 N HCl and .1 N NaOH. Each point is the average of two titrations.

Table 2-1. Formulation (%) of the experimental diets[1]

	Diet			
Item	-NM	+NM	-HM	+HM
Cornstarch	55.60	55.60	50.51	50.51
Fish meal	27.00	27.00	27.00	27.00
Dextrose	10.00	10.00	10.00	10.00
Cellulose	3.00	3.00	3.00	3.00
Soybean oil	3.00	3.00	3.00	3.00
Calcium carbonate	-	-	.74	.74
Dicalcium phosphate	-	-	4.35	4.35
Sodium chloride	.50	.50	.50	.50
Vitamin premix[2]	.20	.20	.20	.20
Mineral premix[3]	.20	.20	.20	.20
Chromic oxide	.50	.50	.50	.50
Formic acid[4]	-	1.18	-	1.18
Total	100.00	101.18	100.00	101.18

[1] Abreviations: NM-normal mineral; HM-high mineral; (-) not supplemented; (+) supplemented with 1% formic acid.

[2] The vitamin premix provided the following per kg diet: 2,100 IU vitamin A; 240 IU vitamin D_3; 13.2 IU vitamin E; .6 mg vitamin K; 480 mg choline; 15 mg niacin; 10.8 mg d-pantothenic acid; 3.6 mg riboflavin; 1.8 mg pyridoxine; 1.2 mg thiamine; .36 mg folacin; .06 mg d-biotin; .018 mg vitamin B_{12}.

[3] The mineral premix provided the following per kg of diet: 480 mg Mg; 96 mg Fe; 96 mg Zn; 6 mg Cu; 3.6 mg Mn; .3 mg Se; .17 mg I.

[4] 85%; BASF Aktiengesellschaft, D-6700 Ludwigshafen, FRG.

Table 2-2. Proximate analysis, calcium, phosphorus and amino acid content (%) of fish meal[1]

Item	
Gross energy[2]	20.06
Dry matter	90.23
Organic matter	72.10
Crude protein	70.81
Ether extract	8.39
Calcium	4.93
Phosphorus	2.86
Ash	18.13
Amino acids	
Indispensable	
Arginine	4.21
Histidine	2.03
Isoleucine	3.28
Leucine	5.28
Lysine	5.98
Phenylalanine	3.16
Threonine	3.13
Valine	3.73
Dispensable	
Alanine	4.44
Aspartic acid	6.75
Glutamic acid	10.08
Glycine	4.30
Serine	2.82
Tyrosine	2.47

[1] Dry matter basis.

[2] MJ per kilogram.

Table 2-3. Proximate analysis, calcium, phosphorus and amino acid content (%) and pH of the experimental diets[1]

	Diet			
Item	-NM[2]	+NM	-HM	+HM
Gross energy[3]	18.61	18.65	17.69	17.58
Dry matter	88.01	87.59	88.78	88.42
Organic matter	80.72	80.31	76.88	76.57
Crude protein	21.36	21.20	20.34	20.13
Ether extract	6.01	6.03	6.06	6.05
Calcium	1.55	1.44	2.71	2.75
Phosphorus	.91	.89	1.78	1.72
Ash	7.29	7.28	11.90	11.85
Amino acids:				
Indispensable				
Arginine	1.14	1.15	1.11	1.11
Histidine	.58	.59	.55	.57
Isoleucine	.93	.90	.88	.88
Leucine	1.52	1.48	1.45	1.46
Lysine	1.64	1.61	1.62	1.60
Phenylalanine	.90	.90	.85	.88
Threonine	.89	.86	.84	.83
Valine	1.05	1.02	1.02	1.00
Dispensable				
Alanine	1.28	1.27	1.23	1.24
Aspartic acid	1.90	1.85	1.82	1.83
Glutamic acid	2.85	2.77	2.70	2.74
Glycine	1.28	1.27	1.21	1.22
Serine	.81	.78	.76	.79
Tyrosine	.64	.60	.59	.62
pH	6.25	4.46	6.13	4.59

[1] Dry matter basis.

[2] Refer to Table 2-1.

[3] MJ per kilogram.

Table 2-4. Apparent ileal digestibilities (%) of dry matter, organic matter, crude protein, ash and amino acids in the experimental diets[1]

	Diet				
Item	-NM[2]	+NM	-HM	+HM	SE[3]
Dry matter	87.5^{a}	84.5^{a}	81.1^{b}	78.6^{b}	.90
Organic matter	90.9^{a}	87.8^{bc}	88.3^{ab}	85.4^{c}	.84
Crude protein	79.5	77.8	79.3	75.2	1.47
Ash	49.6^{a}	43.3^{a}	31.8^{b}	30.6^{b}	2.38
Amino acids:					
Indispensable					
Arginine	88.8	89.8	89.4	87.3	.76
Histidine	85.5	84.6	85.5	83.7	.87
Isoleucine	85.3	86.0	86.6	83.0	1.27
Leucine	85.7	85.7	86.0	82.9	1.23
Lysine	89.0	89.4	90.9	87.7	.86
Phenylalanine	82.4	82.8	83.1	79.7	1.37
Threonine	79.7	78.1	80.3	74.7	1.57
Valine	84.3	84.7	85.7	81.9	1.25
Dispensable					
Alanine	85.1	86.1	86.0	83.7	.98
Aspartic acid	79.8	77.6	80.1	75.8	1.28
Glutamic acid	85.6	86.2	87.6	82.9	1.46
Glycine	78.0	77.0	76.6	73.9	1.25
Serine	79.7	78.2	79.1	75.1	1.56
Tyrosine	84.4	84.5	84.5	81.7	1.56

[1] Least squares means.

[2] Refer to Table 2-1.

[3] Standard errors of the least squares means ($n = 6$).

[a,b] Means within a row with different superscipt letters differ ($P < .05$).

Table 2-5. Apparent fecal digestibilities (%) of energy, dry matter, organic matter, crude protein, ash and amino acids in the experimental diets[1]

	Diet				
Item	-NM[2]	+NM	-HM	+HM	SE[3]
Gross energy	92.9^{a}	90.1^{ab}	88.4^{b}	90.1^{ab}	.90
Dry matter	91.3^{a}	89.8^{a}	84.4^{b}	86.3^{b}	.57
Organic matter	94.3^{a}	91.6^{b}	90.2^{b}	92.0^{b}	.62
Crude protein	88.5	86.9	84.6	86.8	1.01
Ash	55.5^{b}	64.5^{a}	40.3^{c}	43.5^{c}	1.45
Amino acids:					
Indispensable					
Arginine	91.7	92.0	89.7	91.2	.85
Histidine	92.7	91.7	90.3	91.9	.85
Isoleucine	90.1	87.4	86.1	88.6	1.27
Leucine	90.6	88.2	87.0	89.3	1.24
Lysine	92.5	90.9	90.0	92.1	.98
Phenylalanine	89.1	86.9	85.2	88.2	1.24
Threonine	89.8	87.4	85.0	87.4	1.43
Valine	90.5	88.2	87.0	89.3	1.26
Dispensable					
Alanine	89.5	89.0	86.3	89.0	.95
Aspartic acid	89.5	87.5	85.3	87.8	1.06
Glutamic acid	91.8	90.5	88.5	90.6	.97
Glycine	87.6	90.1	85.5	87.6	.99
Serine	88.3	86.6	83.3	86.8	1.37
Tyrosine	88.2	86.6	84.3	86.3	1.34

[1] Least squares means.

[2] Refer to Table 2-1.

[3] Standard errors of the least squares means (n = 6).

[a,b] Means within a row with different superscript letters differ (P < .04).

Table 2-6. Disappearance[1] of dry matter, organic matter, crude protein, ash and amino acids in the large intestine of pigs fed the experimental diets[2]

	Diet				
Item	-NM[3]	+NM	-HM	+HM	SE[4]
Dry matter	34.12^{b}	46.15^{ab}	29.78^{b}	68.56^{a}	7.84
Organic matter	24.28^{b}	26.60^{b}	12.97^{b}	45.02^{a}	5.39
Crude protein	19.22	19.12	10.95	23.41	3.32
Ash	3.72	12.77	9.33	13.62	2.49
Amino acids:					
Indispensable					
Arginine	.33	.24	.03	.43	.11
Histidine	.41	.41	.26	.47	.07
Isoleucine	.45	.12	-.04	.50	.13
Leucine	.75	.36	.13	.94	.21
Lysine	$.56^{a}$	$.24^{ab}$	$-.15^{b}$	$.69^{a}$	.17
Phenylalanine	.59	.37	.19	.75	.14
Threonine	.90	.80	.40	1.06	.15
Valine	.65	.35	.13	.74	.15
Subtotal	4.64	2.89	.95	5.58	
Dispensable					
Alanine	.55	.37	.04	.68	.16
Aspartic acid	1.85	1.83	.95	2.21	.26
Glutamic acid	1.76	1.19	.26	2.13	.40
Glycine	1.35	1.64	1.09	1.67	.22
Serine	.70	.65	.31	.93	.14
Tyrosine	.25	.13	-.02	.29	.11
Subtotal	6.46	5.81	2.63	7.91	

[1] Grams per kilogram dry matter intake; a negative value indicates net synthesis.

[2] Least squares means.

[3] Refer to Table 2-1.

[4] Standard errors of the least squares means (n = 6).

[a,b] Means within a with different superscript letters differ (P < .05).

Table 2-7. The effect of experimental period on the ileal and fecal digestibilities (%) of energy, dry matter, organic matter, crude protein, ash and amino acids[1]

Item	Ileal			Fecal		
Period	1	2	SE[2]	1	2	SE[2]
Gross energy	-	-	-	89.0[b]	91.8[a]	.54
Dry matter	82.2	83.6	.54	87.4	88.5	.35
Organic matter	87.3	88.9	.51	91.2[b]	92.9[a]	.37
Crude protein	76.4[b]	79.5[a]	.89	86.3	87.0	.61
Ash	39.1	38.6	1.44	52.6[a]	49.3[b]	.87
Amino acids:						
Indispensable						
Arginine	87.9[b]	89.8[a]	.46	91.1	91.2	.52
Histidine	84.3	85.4	.53	91.3	92.0	.51
Isoleucine	83.9[b]	86.5[a]	.76	87.3	88.8	.77
Leucine	83.7[b]	86.4[a]	.74	88.3	89.2	.75
Lysine	88.6	90.0	.52	91.2	91.6	.58
Phenylalanine	80.7[b]	83.3[a]	.82	86.7	88.0	.75
Threonine	76.5[b]	79.8[a]	.95	87.1	87.7	.86
Valine	82.8[b]	85.5[a]	.75	88.2	89.2	.76
Dispensable						
Alanine	84.0[b]	86.3[a]	.59	88.1	88.8	.57
Aspartic acid	77.5	79.1	.77	87.1	87.9	.64
Glutamic acid	84.5	86.6	.88	90.2	90.6	.58
Glycine	74.5[b]	77.7[a]	.75	87.8	87.6	.60
Serine	76.6	79.4	.94	86.1	86.4	.83
Tyrosine	82.4	85.2	.94	85.8	86.9	.81

[1] Least squares means.

[2] Standard errors of the least squares means (n = 12).

[a,b] Means within a row within ileal or fecal digestibility with different superscipt letters differ (P < .05).

Table 2-8. The effect of the experimental diets on pH, ammonia and volatile fatty acid concentrations in ileal digesta[1]

	Diet				
Item	-NM[2]	+NM	-HM	+HM	SE[3]
pH	7.66	7.82	7.79	7.71	.09
Ammonia, mM	11.65	8.54	9.53	11.84	.72
VFA, mM					
Acetate	11.65	10.33	11.07	9.83	1.06
Propionate	1.76	1.39	2.05	1.81	.21
Isobutyrate	.12	.10	.17	.09	.05
n-butyrate	.87	.89	1.09	.82	.07
Isovalerate	.26	.23	.31	.14	.06
n-valerate	.07	.06	.14	.09	.02
Total	14.74	13.00	14.84	12.79	1.26
VFA, mol/100 mol					
Acetate	79.70[a]	79.30[a]	74.59[b]	76.18[ab]	.95
Propionate	11.60	11.22	13.64	14.57	1.29
Isobutyrate	.75	.67	1.14	.72	.31
n-butyrate	5.78[c]	6.78[b]	7.46[a]	6.58[b]	.15
Isovalerate	1.74	1.62	2.08	1.20	.26
n-valerate	.43	.41	1.08	.74	.17

[1] Least squares means.

[2] Refer to Table 2-1.

[3] Standard errors of the least squares means (n = 6).

[a,b,c] Means within a row with different superscript letters differ (P < .03).

Table 2-9. The effect of the experimental diets on bacterial cell counts in ileal digesta[1,2]

	Diet				
Item	-NM[3]	+NM	-HM	+HM	SE[4]
Total Lactic Acid Bacteria	7.66	6.63	7.22	7.41	.37
Homofermentative *Lactobacillus*	6.85	6.34	7.13	5.70	.76
Heterofermentative *Lactobacillus*	6.37	5.20	5.48	5.48	1.11
Gram-Positive Anaerobes[5]	9.10	8.91	8.99	9.11	.26
Gram-Negative Anaerobes[6]	7.68	9.80	8.05	5.71	.96
Escherichia coli	7.91	8.31	6.26	6.68	1.05
Lactose Negative Bacteria[7]	6.92	7.65	7.54	6.99	.40
Enterococci[8]	5.85	6.78	6.69	5.95	.24

[1] Least squares means.
[2] Log_{10} of the number of bacterial cells per gram of fresh digesta.
[3] Refer to Table 2-1.
[4] Standard errors of the least squares means (n = 6).
[5] Predominantly from the genus *Eubacterium*.
[6] Family Bacteroidaceae.
[7] Predominantly from the genus *Proteus*.
[8] Genus *Enterococcus*.

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CHAPTER 3

THE EFFECT OF FUMARIC ACID AND SODIUM FUMARATE ON ILEAL AND FECAL AMINO ACID DIGESTIBILITY AND VOLATILE FATTY ACID CONCENTRATIONS IN ILEAL DIGESTA IN EARLY-WEANED PIGS

A. Introduction

Many studies have been carried out to determine the effects of supplementing diets for early-weaned pigs with organic acids. Organic acids have been shown to increase ADG (Henry et al., 1985; Radecki et al., 1988) and gain to feed (G/F) ratio (Falkowski and Aherne, 1984; Giesting and Easter, 1985). One of the proposed reasons for the growth-promoting effect of organic acids is an improvement in the digestibility of nutrients and energy (Kirchgessner and Roth, 1988). However, there is a scarcity of information on the effect of organic acid supplementation on the ileal digestibility of amino acids (AA). With the exception of studies by Mosenthin et al. (1992) with growing-finishing pigs, there is no information on the effect of organic acid supplementation on ileal AA digestibility. These authors reported significant improvements in the ileal digestibilities of several of the indispensable AA when the diets were supplemented with 2% propionic acid.

The primary objective of this study was to investigate the effects of supplementing diets for early-weaned pigs with 1.5 and 3% fumaric acid (FA) or 1.5% sodium fumarate (NaFA) on the ileal and fecal digestibilities of AA. The diet supplemented with 1.5% NaFA would allow for the assessment of whether any effects on digestibility were due to a change in diet pH (H^+ ions from FA) or due to the effect of fumarate on animal and(or) bacterial metabolism. An additional objective of this study was to determine the effects of FA and NaFA on the concentrations of VFA in ileal digesta.

B. Materials and Methods

Animals and Diets

Twelve crossbred barrows (Camborough x Canabrid), weaned at 4 wk, with initial BW 9.3 ± .7 kg, were obtained from the University of Alberta swine herd. The pigs were housed individually in metabolic crates in a temperature-controlled room (25 to 28°C). One week before surgery, the pigs were given ad libitum access to a 20% CP non-medicated starter diet. The diet contained 70.2% wheat, 19.6% soybean meal, 3% canola oil, 3% cornstarch and the remainder consisted of minerals and vitamins according to NRC (1988) standards. Water was freely available from a low-pressure drinking nipple.

A simple T-cannula was surgically placed into the distal ileum, approximately 5 cm anterior to the ileo-cecal sphincter, according to procedures adapted from Sauer et al. (1983) and Li et al. (1993). The pigs were allowed a 7 d recuperation period. During the experiment the pigs were fed three meals, equal amounts, at 0800, 1600 and 2400. Feed intake, at a rate of 5% of the average BW, was determined after the animals were weighed 12 h before the initiation of each period. The pigs were fed 540 g/d during period one and 690 g/d during period two. All pigs usually consumed their meal allowances within 20 min. The average BW of the pigs at the beginning of the first and second periods were 10.7 ± .7 and 14.0 ± 1.0 kg, respectively. The average weight of the pigs at the conclusion of the experiment was 17.4 ± 1.5 kg.

Four diets, based on wheat (cv. Conway) and soybean meal, were formulated to contain 20% CP (N x 6.25). The formulation of the diets is presented in Table 3-1. Wheat and soybean meal were ground through a 3-mm mesh screen before diet formulation. The soybean meal was dehulled and solvent-extracted. Fumaric acid (1.5 and 3%) and NaFA (1.5%) were included in the diets at the expense of cornstarch. Canola oil was included to reduce dustiness, improve palatability and meet NRC (1988) standards for DE. Vitamins, minerals, L-lysine and L-threonine were supplemented to meet or exceed NRC (1988) standards. Chromic oxide (.3%) was included in the diet as marker for the determination of nutrient and energy digestibilities.

The experiment was carried out according to a two-period changeover design (Gill and Magee, 1976). Each experimental period comprised 11 d. Feces were collected on d 6 and 7 and immediately frozen at -28 °C following collection. Ileal digesta were collected on d 8 and 9, beginning at 0800 on d 8 and every other 8 h for a total of 24 h according to Li et al. (1993). Digesta were collected using plastic tubing (width 3 cm; length 20 cm), heat sealed at one end, and fastened to the barrel of the cannula by aid of a Velcro-elastic strap. Before the tubing was attached, digesta were removed from the inside of the barrel of the cannula with a spatula. During collection, care was taken to ensure that the barrel of the cannula did not become blocked with digesta. The digesta were immediately frozen at -28°C after collection. The tubing was emptied when it was approximately one third to one half full with digesta. Before reattachment, 5 mL of formic acid (10% vol/vol) was added to minimize bacterial activity in collected digesta. On d 11, digesta for pH measurement and determination of VFA concentrations were collected from 0800 to 1600. The procedure used was similar to the protocol used on d 8 and 9. To avoid diluting the digesta, formic acid was not added to the collection tube. Every 2 h of collection was considered one sampling time. Digesta were collected from 0 to 2 h, 2 to 4 h, 4 to 6 h, and 6 to 8 h postprandial. During each 2 h collection, the tubings were frequently emptied into polyethylene bottles which were kept in an ice bath. The bottles were tightly capped. New collection tubing was used each time after these were emptied, in order to minimize the risk of sample contamination and accumulation of oxidation products. The pH of the digesta in the collection bottles was measured[1] after every 2 h collection. A 15-mL polypropylene tube was filled with digesta and centrifuged at 400 x g for 10 min. After centrifugation the tubes were put into ice and 2 mL of supernatant was transferred to a second tube which contained .5 mL of 25% (vol/vol) ortho-phosphoric acid (Khorasani et al., 1992). The tubes were tightly capped, mixed with a vortex and immediately frozen at -28°C. The addition of ortho-phosphoric acid served to minimize bacterial activity; the pH of the supernatant was approximately two.

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[1] Beckman® model 4500, Beckman Instruments, Irvine, CA.

Chemical and Statistical Analysis

Chemical analyses of ingredients, diets, digesta, and feces were carried out according to procedures described in Chapter two. Additionally, analyses for NDF, ADF, cellulose, and hemicellulose were carried out according to principles outlined by Goering and van Soest (1970).

The method of French and Kennelly (1990), for determining VFA concentration in ruminal fluid, was adapted to allow for the determination of VFA concentrations in ileal digesta. An internal standard solution was prepared by weighing .4 g of 4-methyl-n-valeric acid into a 100-mL volumetric flask containing approximately 10 mL distilled water. Thereafter, 20 mL of 25% (vol/vol) ortho-phosphoric acid, and 6 mL of distilled ethanol were added. The flask was brought up to volume with distilled water. The addition of ethanol increased the solubility of 4-methyl-n-valeric acid, which is only slightly soluble in water (CRC, 1980). Acid was added to ensure that the pH in the internal standard solution was approximately the same as that of the sample. A standard VFA solution was prepared similarly: .6 g of acetic, .1 g each of propionic, isobutyric, n-butyric, isovaleric, n-valeric, 4-methyl-n-valeric, and n-caproic acid, 40 mL 25% (vol/vol) ortho-phosphoric acid, 3 mL distilled ethanol, and distilled water in a 200-mL volumetric flask. The samples (2.5 mL) were thawed at room temperature (21 °C) and .2 mL of the internal standard was added. The sample was transferred to two 1.5-mL microcentrifuge tubes and centrifuged[2] for 10 min at 12,400 x g to ensure that all insoluble matter was removed, including chromic oxide. Concentrations of VFA in the supernatant were determined using a Varian[3] Model 3700 gas chromatograph with a flame-ionization detector and capillary column[4]. The column was operated at 120°C internal temperature programmed at 10°C/min to 180°C. Helium was used as carrier gas at a flow rate of 1 mL/min. Split injection was used; the split ratio was 30 to 1. Peaks were recorded and integrated with the Shimadzu EZchrom™ Chromatography Data System (version 2.12)[5].

Statistical analysis were performed as described in Chapter two. Additionally, linear and quadratic effects of supplementing diets with 1.5, and 3% FA were analyzed according to the orthogonal polynomial regression procedure (Steel and Torrie, 1980). The pH and VFA concentrations in ileal digesta were analyzed as repeated measures (Gill and Hafs, 1971) using the GLM procedure of SAS (1988). However, test for sphericity (P > .2) for some traits indicated that F-values from split-plot analysis were valid (SAS, 1991). The error term period x diet x animal was used to test the effects of period, diet, and pig in the whole plot. Time and diet x time were included in the spit-plot. The repeated statement was used to evaluate linear, quadratic, and cubic effects (SAS, 1991). Time least squares means were compared using Fisher's LSD procedure (Milliken and Johnson, 1984), if a significant F-value was detected (P < .05).

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[2] Eppendorf 5412, Brinkmann Instruments Inc., Wesbury, NY.

[3] Varian Associates, Sunnyvale, CA.

[4] Stabilwax-DA® (Crossbonded® Carbowax® -PEG for acids), 30 m long, .25 mm i.d., .5 µm film thickness, Restek Corporation, Bellefonte, PA.

[5] Shimadzu Scientific Instruments Inc., 7102 Riverwood Drive, Columbia, MD.

C. Results and Discussion

The pigs remained healthy and consumed their meal allowances throughout the experiment. Post-mortem examinations, carried out at the conclusion of the experiment, revealed no intestinal adhesions.

The chemical composition of the wheat and soybean meal is presented in Table 3-2. The chemical composition of the experimental diets is presented in Table 3-3. The diets were nearly the same in chemical composition. The differences in the GE contents between the diets (maximum 1.2%) can be explained by the replacement of cornstarch with either FA or NaFA. The diets were nearly isoenergetic The GE contents of FA (11.51) and NaFA (8.33 kJ/g), were similar to the heats of combustion reported by CRC (1980) which were, 11.50 and 8.34 kJ/g, respectively. The high ash content of the diet supplemented with NaFA can be explained by the high Na content (14.37%) of NaFA. The pH of the control diet was 6.33. Supplementation with 1.5 and 3% FA decreased the pH of the diets to 4.35 and 3.93, respectively. The pH of the NaFA diet was 6.30, nearly similar to that of the control diet. Risley et al. (1992) reported a decrease in diet pH (6.42 vs 4.70) when a starter diet was supplemented with 1.5% FA.

The apparent ileal and fecal digestibility coefficients of GE, DM, CP, and AA in the experimental diets are presented in Tables 3-4 and 3-5, respectively.

The ileal CP and AA digestibilities are within the range of values reported by Sauer and Ozimek (1986). Supplementation with 3% FA decreased ($P < .04$) the ileal digestibilities of GE and CP. There was a trend ($P < .09$) for a decrease in the digestibility of DM. The decreases were linear ($P < .01$) with increasing FA level. A decrease in digestibility in response to supplementation of diets with organic acids has not been reported before in the literature. Giesting and Easter (1991) found no effect ($P > .10$) of supplementation with 2% FA to corn-soybean meal and corn-dried skim milk diets on the ileal digestibilities of DM and N. Mosenthin et al. (1992) also reported that supplementation with 2% propionic acid did not affect ($P > .05$) the ileal digestibilities of DM, OM, GE or CP.

Supplementation with 3% FA decreased (P < .04) the digestibilities of arginine, glycine and tyrosine. The decreases were linear (P < .02) with increasing FA level. The decreases tended (P < .10) to be linear for alanine, glutamic acid, leucine, phenylalanine and serine. For tyrosine the decrease was quadratic (P = .05) with increasing FA level. There was a trend for a decrease (P < .06) in phenylalanine digestibility. The digestibilities of the other AA were not affected (P > .15) by FA supplementation. In contrast, Mosenthin et al. (1992) reported that supplementation with 2% propionic acid increased (P < .05) the apparent ileal digestibilities of several AA.

The decrease in the ileal digestibilities of GE, DM, CP, and AA could have been due, in part, to increased microbial activity in the small intestine. Approximately 30% of N in ileal digesta originates from bacterial N (Dierick et al., 1983; Poppe et al., 1983). Therefore, bacterial N metabolism may influence the apparent ileal digestibility. If energy is the limiting factor for microbial growth in the small intestine, supplementation of a readily available energy source to bacteria, like fumarate, would be expected to increase bacterial populations. The animals, fed the diet supplemented with 3% FA, consumed 16.2 and 20.7 g/d of FA during period one and two, respectively. Bacterial metabolism of fumarate can also occur. Fumarate can be reduced to succinate by fumarate reductase and this reaction is part of the succinate-propionate pathway (Gottschalk, 1986). The fumarate reductase enzyme system occurs in Propionobacterium, Bacteroides, Veillonella, Peptostreptococcus, Ruminococcus, Succinivibrio, Selenomonas, Clostridium and Escherichia coli. All these organisms can be found in the gastrointestinal tract (Gottschalk, 1986; Drasar, 1988). Risley et al. (1991, 1992), reported that supplementation with 1.5% FA increased ($P < .05$) the concentration of fumarate in the stomach and jejunum but not in the cecum or lower colon. Notwithstanding, there could have been other effects. For instance, FA may be less readily aborbed than the end products of cornstarch digestion, namely glucose. If this is the case, more indigestible matter would be collected at the ileum and therefore digestibilties would be lower.

The significance of these results is difficult to assess. Giesting et al. (1991) reported an interaction ($P < .05$) between FA and sodium bicarbonate for ADG and G/F. Pigs fed diets supplemented with both FA and sodium bicarbonate had higher ADG and G/F. Giesting et al. (1991) hypothesized that this may have been due to prevention of metabolic acidosis by sodium bicarbonate and(or) an effect of FA on intermediary metabolism resulting in improved energy or amino acid utilization (Giesting et al., 1991). However, Eidelsburger et al. (1992a) reported no effect ($P > .05$) of supplementation of 1.8% FA on acid-base-status.

The uptake of fumarate and citrate from the lumen of the proximal jejunum occurs by a common Na^+-gradient dependent mechanism in both calves (Wolffram et al., 1990) and pigs (Wolffram et al., 1992). Fumaric acid could contribute to the formation of ATP and possibly have a subsequent N-sparing effect on glucogenic AA, such as glutamine. This could help facilitate the rapid developmental changes that are occurring in the digestive system of the early-weaned pig. For example, when acetate was supplemented at a rate of 5 and 10% of ME intake, an average N-sparing effect of 32.9 mg N/g of acetate was observed (Imoto, 1983).

The apparent ileal digestibility of arginine was relatively high, whereas the ileal digestibilities of threonine and glycine were relatively low. Possible reasons for the relatively low and high apparent ileal AA digestibilities were discussed in Chapter two.

There was no effect ($P > .4$) of supplementation of diets for early-weaned pigs with FA or NaFA on the apparent fecal digestibilities of GE, DM, CP and AA. Similar results were reported by Falkowski and Aherne (1984) and Eidelsburger et al. (1992b). On the other hand, Paullauf et al. (1987) observed an improvement ($P < .05$) in the apparent fecal digestibilities of CP, OM, and GE from the supplementation of 1.5 and 3% FA. The results of this study confirm the findings of Sauer and Ozimek (1986). The ileal analysis method is more sensitive than the fecal analysis method for determining differences among diets or differently treated diets. This study, as was the case in other studies (e.g. Sauer et al., 1977; Li et al., 1993) illustrates the modifying and apparent equalizing effect of the microflora in the large intestine on AA digestibilities.

The net disappearances of GE, DM, CP, and AA in the large intestine, expressed as grams per kilogram DMI, is presented in Table 3-6. The supplementation of diets with 3% FA increased (P < .04) the disappearances of GE, DM and glycine. There was a trend (P < .10) for increases in the disappearances of CP and glutamic acid. The increases in the disappearances of GE, DM and glycine were linear (P < .03) as the level of FA supplementation was increased. There was a trend (P < .08) for increases in the disappearances of CP, arginine and glutamic acid. The increases in disappearances were due to the lower ileal digestibilities, subsequently, the additional nutrients entering the large intestine were digested and fermented by the microbes. Once more, these results illustrate the modifying and apparent equalizing effect of bacterial N metabolism. A net synthesis of AA was not observed for any of the dietary treatments. For each diet, there was a larger disappearance of the dispensable than of the indispensable AA which was also reported by Li et al. (1993) and in Chapter two.

The apparent ileal as well as fecal digestibilities of GE, DM, CP and AA for each experimental period are presented in Table 3-7. The ileal digestibility of glycine was higher (P

= .02) in period two than in period one. The digestibility of CP tended to be higher (P = .07). There was no effect (P > .10) of experimental period on the apparent fecal digestibilities of GE, DM, CP, and AA.

The pH and VFA concentrations in ileal digesta are presented in Table 3-8. There was no effect (P > .12) of diet on the pH of ileal digesta or acetate and propionate concentrations. For the diets supplemented with 1.5 and 3% FA, the concentration of n-butyrate in ileal digesta was increased (P < .05). This increase was quadratic (P = .04) with increasing FA supplementation. The total VFA concentration in digesta from pigs fed diets supplemented with FA or NaFA tended to be higher (P < .08) than for pigs fed the control diet. These increases may have been due to increased bacterial activity in the small intestine. Similar results were found by Sutton et al. (1991); supplementation with 1.5% FA increased (P < .05) the acetate, propionate, and total VFA concentrations in the cecum. There was no effect of dietary treatment (P > .16) on acetate, propionate or butyrate expressed as mol/100 mol in ileal digesta. In addition, there were no period effects (P > .14) on VFA concentrations in ileal digesta.

The pH and VFA concentrations in ileal digesta collected at different times postprandial are presented in Table 3-9. The pH and VFA concentrations exhibited a quadratic or cubic relationship within 8 h postprandial. The pH of ileal digesta from 0 to 2 h was higher (P = .0001) than the pH from 2 to 8 h postprandial. Acetate concentration in ileal digesta peaked (P < .03) at 4 to 6 h postprandial. Propionate concentration was highest (P = .0001) at 0 to 2 h postprandial and gradually declined during the remaining 6 h postprandial. Butyrate concentration exhibited the same trend as propionate and was lowest (P < .01) 6 to 8 h postpradial. Total VFA concentration tended (P < .08) to peak at 4 to 6 h postprandial. Rérat et al. (1987) reported that absorption of VFA was greater (P < .05) at 5 to 10 h than 0 to 4 h postprandial. The observed increases in VFA concentrations and subsequent absorption at 5 h postprandial corresponded to the arrival of the first indigestible fractions of the diet in the large intestine (Keys and de Barthe, 1974). The same trend was observed in this study; total VFA concentration peaked at 4 to 6 h postprandial. This time coincides with maximal digesta flow (V.M. Gabert, and W.C. Sauer, unpublished data) which indicates that the majority of the indigestible fraction of the meal reached the distal ileum and fermentation of carbohydrates has increased. At all sampling times, acetate made up the highest percentage of the VFA (80.05 to 89.03%). The percentage of acetate was lowest (P = .0001) at 0 to 2 h after feeding. The percentage of propionate was highest (P = .0001) during 0 to 2 h postprandial. The percentage of n-butyrate followed the same pattern as propionate and was highest (P < .01) from 0 to 2 h postprandial.

In summary, the supplementation of diets for early-weaned pigs with 3% FA decreased (P < .05) the apparent ileal digestibilities of GE, CP and some of the AA. However, supplementation of diets with 1.5% FA or 1.5% NaFA did not affect (P > .05) the apparent digestibilities of GE, CP or AA. Supplementation of diets for early-weaned pigs with FA did not affect (P > .12) the pH of ileal digesta, or the concentration of acetate or propionate. Supplementation of diets with 1.5 and 3% FA increased (P < .05) the concentration of n-butyrate in ileal digesta. It appears, from this study, that supplementation of diets with FA affects microbial activity in the small intestine.

Table 3-1. Formulation (%) of the experimental diets[1]

Item	Control	1.5% FA	3.0% FA	1.5% NaFA
Wheat	69.71	69.71	69.71	69.71
Soybean meal	19.72	19.72	19.72	19.72
Canola oil	3.00	3.00	3.00	3.00
Calcium carbonate	1.00	1.00	1.00	1.00
Calcium phosphate[2]	1.65	1.65	1.65	1.65
Chromic oxide	.30	.30	.30	.30
Vit.-min. premix[3]	1.00	1.00	1.00	1.00
Iodized salt	.40	.40	.40	.40
L-Threonine (98%)	.01	.01	.01	.01
L-Lysine (78%)	.21	.21	.21	.21
Cornstarch	3.00	1.50	-	1.50
Fumaric acid[4]	-	1.50	3.00	-
Sodium fumarate[5]	-	-	-	1.50
Total	100.00	100.00	100.00	100.00

[1] Abbreviations: FA - fumaric acid; NaFA - sodium fumarate.

[2] Dibasic; $CaHPO_4 \cdot 2H_2O$.

[3] The vitamin-mineral premix provided the following per kg diet: 5000 IU vitamin A; 500 IU vitamin D; 40 IU vitamin E; .03 mg vitamin B_{12}; 12 mg riboflavin; 45 mg niacin; 25 mg d-pantothenic acid; 600 mg choline; .2 mg d-biotin; .2 mg folic acid; 150 mg Fe; 20 mg Mn; 120 mg Zn; 125 mg Cu; .2 mg I; .3 mg Se.

[4,5] Crystalline, Sigma Chemical Co., St. Louis, MO.

Table 3-2. Proximate analysis and amino acid content (%) of wheat and soybean meal[1]

Item	Wheat	Soybean meal
Gross energy[2]	19.00	19.72
Dry matter	87.34	89.34
Crude protein	17.54	51.46
Neutral-detergent fiber	12.67	7.04
Acid-detergent fiber	3.15	3.56
Cellulose	1.99	3.39
Hemicellulose	9.53	3.48
Ether Extract	1.87	.77
Ash	1.77	6.19
Amino acids:		
Indispensable		
Arginine	.72	3.18
Histidine	.39	1.23
Isoleucine	.61	2.03
Leucine	1.16	3.36
Lysine	.47	3.12
Phenylalanine	.79	2.15
Threonine	.51	1.84
Valine	.75	2.01
Dispensable		
Alanine	.63	1.91
Aspartic acid	.86	5.02
Glutamic	5.63	8.66
Glycine	.69	1.90
Serine	.80	2.22
Tyrosine	.37	1.47

[1] Dry matter basis.

[2] MJ per kilogram.

Table 3-3. Proximate analysis, amino acid content (%) and pH of the experimental diets[1]

	Diet			
Item	Control	1.5% FA	3.0% FA	1.5% NaFA
Gross energy[2]	18.84	18.66	18.61	18.70
Crude protein	22.58	23.11	22.91	22.48
Dry matter	89.65	89.78	90.27	89.84
NDF[3]	9.99	9.98	9.93	9.97
ADF[4]	2.83	2.83	2.81	2.83
Cellulose	2.02	2.02	2.01	2.01
Hemicellulose	7.15	7.14	7.10	7.13
Ash	6.22	6.09	6.12	6.80
Ether extract	4.96	4.98	4.56	4.59
Amino acids:				
Indispensable				
Arginine	1.18	1.14	1.17	1.16
Histidine	.49	.50	.49	.51
Isoleucine	.90	.88	.90	.87
Leucine	1.50	1.46	1.47	1.46
Lysine	1.14	1.08	1.08	1.11
Phenylalanine	.96	.92	.92	.88
Threonine	.75	.72	.72	.73
Valine	.93	.92	.93	.90
Dispensable				
Alanine	.82	.80	.83	.80
Aspartic acid	1.70	1.68	1.71	1.68
Glutamic acid	5.43	5.52	5.50	5.56
Glycine	.85	.84	.85	.85
Serine	.98	.98	.99	.99
Tyrosine	.56	.57	.51	.56
pH	6.33	4.35	3.93	6.30

[1] Dry matter basis.
[2] MJ per kilogram.
[3] Neutral-detergent fiber.
[4] Acid-detergent fiber.

Table 3-4. Apparent ileal digestibilities (%) of energy, dry matter, crude protein and amino acids in the experimental diets[1]

	Diet				
Item	Control	1.5% FA	3.0% FA	1.5% NaFA	SE[2]
Gross energy[3]	73.0[a]	71.3[ab]	69.0[b]	71.8[a]	.75
Dry matter	71.4	69.7	67.6	70.1	.86
Crude protein[3]	80.3[a]	79.9[a]	76.2[b]	79.2[a]	.57
Amino acids:					
Indispensable					
Arginine[3]	87.2[a]	85.8[a]	82.7[b]	85.6[a]	.78
Histidine	85.6	86.1	84.3	84.7	.73
Isoleucine	85.1	84.6	83.7	83.6	.61
Leucine	85.3	84.4	83.2	83.6	.73
Lysine	82.6	81.6	80.8	80.7	.90
Phenylalanine	85.0	83.8	82.0	82.0	.72
Threonine	75.5	75.5	74.0	73.7	1.11
Valine	82.4	82.2	80.8	80.3	.76
Dispensable					
Alanine	76.2	76.4	73.5	74.4	.95
Aspartic acid	79.8	80.4	79.0	79.7	.65
Glutamic acid	90.8	90.8	89.1	90.3	.50
Glycine[3]	67.7[a]	66.5[a]	58.0[b]	66.5[a]	1.55
Serine	80.4	80.5	78.6	79.2	.64
Tyrosine[4]	83.0[a]	83.7[a]	79.5[b]	81.9[ab]	.82

[1] Least squares means.

[2] Standard errors of the least squares means (n = 6).

[3] Linear effect ($P < .01$).

[4] Quadratic effect ($P < .05$).

[a,b] Means within a row with different superscript letters differ ($P < .04$).

Table 3-5. Apparent fecal digestibilities (%) of energy, dry matter, crude protein and amino acids in the experimental diets[1]

	Diet				
Item	Control	1.5% FA	3.0% FA	1.5% NaFA	SE[2]
Gross energy	86.6	86.4	85.7	85.9	.69
Dry matter	87.5	87.4	87.1	86.8	.54
Crude protein	89.2	89.0	88.4	87.8	.88
Amino acids:					
Indispensable					
Arginine	92.6	91.5	91.4	90.6	.80
Histidine	91.7	90.9	90.7	90.3	.86
Isoleucine	88.0	87.1	86.5	85.8	1.03
Leucine	89.3	88.3	88.2	87.3	.93
Lysine	85.8	82.2	83.1	82.2	1.71
Phenylalanine	90.0	89.0	89.8	88.3	1.26
Threonine	85.5	84.3	83.6	83.1	1.27
Valine	87.0	86.4	85.8	84.5	1.14
Dispensable					
Alanine	83.9	82.1	82.1	80.8	1.44
Aspartic acid	87.5	86.4	86.3	85.6	1.13
Glutamic acid	95.4	94.9	94.9	94.6	.46
Glycine	87.3	86.4	86.3	85.6	1.08
Serine	90.5	90.1	90.0	89.2	.77
Tyrosine	87.4	86.5	84.8	85.1	1.13

[1] Least squares means.
[2] Standard errors of the least squares means (n = 6).

Table 3-6. Disappearance[1] of energy, dry matter, crude protein and amino acids in the large intestine of pigs fed the experimental diets[2]

	Diet				
Item	Control	1.5% FA	3.0% FA	1.5% NaFA	SE[3]
Gross energy[4,5]	2.56[b]	2.82[ab]	3.11[a]	2.64[b]	.11
Dry matter[5]	144.32[b]	158.68[ab]	176.45[a]	150.25[b]	5.39
Crude protein	19.91	20.98	28.04	19.16	.88
Amino acids:					
Indispensable					
Arginine	.64	.65	1.02	.58	.12
Histidine	.30	.24	.31	.28	.05
Isoleucine	.26	.22	.26	.19	.09
Leucine	.60	.57	.72	.54	.14
Lysine	.37	.06	.25	.17	.19
Phenylalanine	.48	.48	.72	.55	.11
Threonine	.75	.64	.70	.68	.09
Valine	.43	.39	.47	.37	.10
Subtotal	3.83	3.25	4.45	3.36	
Dispensable					
Alanine	.64	.45	.71	.51	.12
Aspartic acid	1.31	1.00	1.24	.98	.18
Glutamic acid	2.50	2.28	3.20	2.38	.23
Glycine[5]	1.67[b]	1.68[b]	2.40[a]	1.62[b]	.17
Serine	.99	.94	1.13	.99	.09
Tyrosine	.24	.16	.27	.18	.07
Subtotal	7.35	6.51	8.95	6.66	

[1] Grams per kilogram dry matter intake.

[2] Least squares means.

[3] Standard errors of the least squares means (n = 6).

[4] Kilojoules per kilogram dry matter intake.

[5] Linear effect (P < .03).

[a,b] Means within a row with different superscript letters differ (P < .04).

Table 3-7. The effect of experimental period on the ileal and fecal digestibilities (%) of energy, dry matter, crude protein and amino acids[1]

Item	Ileal			Fecal		
Period	1	2	SE[2]	1	2	SE[2]
Gross energy	72.0	70.6	.45	86.3	86.0	.42
Dry matter	70.5	68.9	.52	87.5	86.9	.32
Crude protein	78.4	79.4	.34	88.1	89.1	.53
Amino acids:						
Indispensable						
Arginine	85.0	85.6	.47	91.1	92.0	.48
Histidine	85.2	85.1	.44	90.3	91.4	.52
Isoleucine	84.4	84.1	.37	86.1	87.6	.62
Leucine	84.2	84.0	.44	87.6	88.9	.56
Lysine	81.0	81.8	.54	82.2	84.4	1.03
Phenylalanine	83.4	83.0	.44	88.2	90.4	.76
Threonine	74.1	75.2	.67	83.2	85.1	.76
Valine	81.5	81.3	.46	85.1	86.8	.69
Dispensable						
Alanine	74.8	75.5	.58	81.2	83.3	.87
Aspartic acid	79.4	80.1	.39	85.6	87.3	.68
Glutamic acid	90.4	90.1	.30	94.7	95.2	.28
Glycine	62.7[b]	66.6[a]	.93	85.6	87.2	.65
Serine	79.6	79.8	.38	89.4	90.5	.47
Tyrosine	81.9	82.2	.49	85.0	86.9	.68

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[1] Least squares means.

[2] Standard errors of the least squares means (n = 12).

[a,b] Means within a row within ileal or fecal digestibility with different superscript letters differ (P < .02).

Table 3-8. The effect of the experimental diets on pH and volatile fatty acid concentrations in ileal digesta[1]

Item	Diet				
	Control	1.5% FA	3.0% FA	1.5% NaFA	SE[2]
pH	6.65	6.68	6.55	6.51	.07
VFA, mM[3]					
Acetate	16.84	26.21	25.59	20.01	2.54
Propionate	1.98	3.00	1.93	2.05	.32
n-butyrate[4]	.80[b]	1.79[a]	1.53[a]	.85[b]	.19
Total	19.61	31.00	29.05	22.91	2.68
VFA, mol/100 mol					
Acetate	85.04	84.96	88.37	87.11	1.97
Propionate	11.36	9.43	6.68	9.24	1.87
n-butyrate	3.60	5.61	4.95	3.65	.57

[1] Least squares means.

[2] Standard errors of the least squares means (n = 24).

[3] Only trace amounts of isobutyrate, isovalerate, n-valerate, and n-caproate were detected.

[4] Quadratic effect (P = .04).

[a,b] Means within a row with different superscript letters differ (P < .05).

Table 3-9. The effect of sampling time on pH and volatile fatty acid concentrations in ileal digesta[1,2]

	Hours Postprandial				
Item	0 to 2	2 to 4	4 to 6	6 to 8	SE[3]
pH[4]	6.80[a]	6.50[b]	6.55[b]	6.56[b]	.07
VFA, mM					
Acetate	21.07[b]	21.91[ab]	24.82[a]	20.35[b]	1.18
Propionate[4]	3.70[a]	2.10[b]	1.70[b]	1.46[b]	.27
n-butyrate	1.51[a]	1.16[ab]	1.36[a]	.93[b]	.12
Total	26.27	25.18	27.88	22.74	1.45
VFA, mol/100 mol					
Acetate[5]	80.05[b]	87.44[a]	88.61[a]	89.03[a]	.72
Propionate[5]	14.36[a]	8.48[b]	6.92[b]	7.23[b]	.57
n-butyrate[5]	5.59[a]	4.08[b]	4.48[b]	3.74[b]	.30

[1] Least squares means.

[2] Only trace amounts of isobutyrate, isovalerate, n-valerate, and n-caproate were detected.

[3] Standard errors of the least squares means (n = 24).

[4] Quadratic effect (P < .002).

[5] Cubic effect (P < .03).

[a,b] Means within a row with a different superscript letter differ (P < .05).

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CHAPTER 4

THE EFFECT OF OLIGOSACCHARIDES AND LACTITOL ON THE ILEAL DIGESTIBILITIES OF AMINO ACIDS, CARBOHYDRATES AND BACTERIAL POPULATIONS AND METABOLITES IN THE DIGESTIVE TRACT OF EARLY-WEANED PIGS

A. Introduction

Early-weaning of pigs frequently results in growth suppression and diarrhea. This condition may persist for several days, even weeks, and result in mortality. Nondigestible oligosaccharides (e.g., fructooligosaccharides and transgalactosylated oligosaccarides) and lactitol are readily fermented and promote the growth of lactic acid bacteria, e.g., *Bifidobacterium* and *Lactobacillus*. These bacteria suppress the growth of pathogenic and putrefactive bacteria and may reduce the incidence of diarrhea (van Velthuijsen, 1979; Tanaka et al., 1983; Hidaka et al., 1986; Modler et al., 1990; Roberfroid et al., 1993). Supplementation of diets with oligosaccharides or lactitol can also increase Ca^+ absorption (Ammann et al., 1988; Levrat et al., 1991). Supplementation of diets with fructooligosaccharide has been shown to increase epithelial cell proliferation in the cecum and colon of neonatal pigs (Howard et al., 1993). However, there is a scarcity of information on the effect of oligosaccharides and lactitol on nutrient digestion and activity of the microflora in the small intestine.

The objectives of this study were to investigate the effects of the supplementation of diets for early-weaned pigs with oligosaccharides or lactitol on the ileal digestibilities of CP, amino acids (AA), and monosaccharides. An additional objective was to investigate the effect of supplementing diets with oligosaccharides and lactitol on the concentration of microbial metabolites and bacterial populations in digesta collected from the distal ileum.

B. Materials and Methods

Animals and Diets

Twelve crossbred[1] barrows, weaned at 4 wk, initial BW 9.1 ± .7 kg, were obtained from a local pig farm. The pigs were housed in stainless steel metabolic crates in a temperature controlled room (25-27 °C). An 18% CP non-medicated starter diet (Sauer et al., 1983) was fed 1 wk before surgery. The pigs had ad libitum access to feed and water. A simple T-cannula was surgically placed into the distal ileum, approximately 5 cm anterior to the ileo-cecal sphincter, according to procedures adapted from Sauer et al. (1983) and Li et al. (1993). Modifications in procedures and pre- and post-operative care were described previously (Chapter two).

The pigs were allowed a 7 d recuperation period. During the experiment the pigs were fed at 0530, 1330 and 2130. Feed intake, at a rate of 4.5% of BW, was determined after the pigs were weighed the day before the start of each experimental period; 453 ± 38 g/d during period one and 559 ± 48 g/d during period two. The average BW of the pigs at the beginning of the first and second experimental periods were 10.1 ± .8 kg and 12.4 ± 1.1, respectively. The average BW of the pigs at the conclusion of the experiment was 13.8 ± 1.4 kg. The pigs were given ad libitum access to water before and after feeding. The pigs usually consumed their meal allowances within 20 min.

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[1] The pigs were products from a cross breeding system involving the following breeds: German Landrace, Duroc, Piétrain, Belgian Landrace and Hampshire. Hülsenberger Swine Breeding Program, H. Wilhelm Schaumann, Forschungszentrum für Tierernährung und Tierzucht, Versuchsgut Hülsenberg D-2362 Wahlstedt, FRG.

Four diets, based on wheat, barley and soybean meal, were formulated to contain 18% CP (N x 6.25). The formulation of the experimental diets is presented in Table 4-1. The wheat, barley and soybean meal were ground through a 3-mm mesh screen before diet formulation. The oligosaccharides and lactitol were obtained from Lohmann Tierernährung GmbH, Cuxhaven, Germany. The galactooligosaccharide (GAO) product contained 40% (as-fed) transgalactosylated oligosaccharides (TOS) with a composition of galactose-(galactose)$_n$-glucose where n = 1 to 4 linked through β-(1→6), (1→4) and (1→3) linkages (Tanaka et al., 1983). The glucooligosaccharide (GUO) product contained 23% (as-fed) glucooligosaccharides which consisted primarily of di-, tri- and tetrasaccharides synthesized by condensation of D-glucose by β-glucosidase (Ajisaka et al., 1987; Fujimoto et al., 1988). The GAO, GUO and lactitol diets contained .2% TOS, .2% GUO and 1% lactitol (4-O-β-D-galactopyranosyl-D-sorbitol), respectively, included at the expense of cornstarch. Soybean oil was included to reduce dustiness and meet NRC (1988) standards for DE. Vitamins, minerals, L-lysine, DL-methionine, and L-threonine were supplemented to meet or exceed NRC (1988) standards. Chromic oxide (.5%) was included in the diet as marker for the determination of nutrient digestibilities.

The experiment was carried out according to a two-period changeover design (Gill and Magee, 1976). Each experimental period comprised 9 d; 6 d adaptation followed by a 3 d collection. Ileal digesta for the determination of pH, VFA and ammonia concentrations were collected on d 7 starting at 0530, from 0 to 1.5, 3 to 4.5 and 6 to 7.5 h postprandial, respectively. Samples for bacterial counts were taken from digesta collected from 0 to 1.5 and 3 to 4.5 h postprandial. Digesta were collected by the use of plastic tubing (width: 3 cm; length 20 cm), heat-sealed at one end and fastened to the barrel of the cannula by a Velcro-elastic strap. Before the tubing was attached, digesta were removed from the barrel of the cannula with a spatula. A 5.5 mL glass vial was filled with digesta (there was no headspace) and capped when approximately 30 mL of digesta were collected from each animal. The remainder was immediately processed in the laboratory as described under chemical analysis. The vials were vacuum-sealed in a polyethylene bag with a CO_2-generating and O_2-binding anaerobic pack[2] and delivered to the Institute of Medical Microbiology, Ludwig-Maximilians University, Munich, Germany. Ileal digesta for the other analyses were collected for a total of 24 h. The collection was initiated on d 8, from 0530 to 1330, and every other 8 h according to Li et al. (1993). Digesta were immediately frozen at -30 °C. The tubing was emptied when it contained 20 to 40 mL of digesta. Before the tubing was reattached to the barrel of the cannula, 5 mL of copper sulfate (1% wt/vol) was added to minimize bacterial activity (McBurney and Thompson, 1990).

Following the completion of the experiment, the cannulas were removed from the pigs. The pigs were anaesthetized and given antibiotics using the same protocol as for surgery (Chapter two). The cannula was pulled out of the fistula and a single buried suture of silk was inserted around the fistula and tightened. A rolled piece of gauze (3 x 3 cm) was placed between the knot and the skin. The area around the fistula was washed twice each day to minimize irritation from leaking digesta. The fistula closed within 2 to 3 wk; the pigs were slaughtered at 100 kg.

Chemical and Statistical Analysis

Chemical analyses of ingredients, diets, and freeze-dried digesta were carried out according to procedures described in Chapters two and three. Additionally, the ingredients, oligosaccharides, diets and freeze-dried digesta were analyzed for monosaccharides according to Blakeney et al. (1983) and Kraus et al. (1990) modified according to Lien and Sauer (1993). Approximately 50 mg of sample was hydrolyzed in 3 mL of 12 M H_2SO_4 (72% wt/wt) at 21 °C for 1 h followed by hydrolysis in 12 mL of 3 M H_2SO_4 at 110 °C for 1 h. Sequential hydrolysis curves were plotted for the GAO and GUO following hydrolysis at 110 °C for 15, 30, 45, 60, 75 and 90 min, respectively. After the addition of the internal standards, namely myo-inositol and N-methylglucamine (N-methyl-2-amino-2-deoxy-D-glucose), the alditol acetate derivatives of the monosaccharides were quantified by a Varian 3400 gas chromatograph using the conditions outlined by Lien and Sauer (1993).

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[2] Anaerocult®; contains kieselgur, iron powder, citric acid and sodium carbonate. E. Merck, Postfach 41 91, D-6100 Darmstadt 1, FRG.

The pH, ammonia and VFA concentrations as well as the bacterial populations in ileal digesta were determined according to procedures described in Chapter two.

Statistical analysis was performed as described in Chapters two and three.

C. Results and Discussion

The pigs remained healthy and consumed their meal allowances throughout the experiment. No scouring was observed. Post-mortem examinations, carried out after the pigs were slaughtered at 100 kg, revealed no intestinal adhesions and other abnormalities. The chemical composition of barley, wheat, and soybean meal is presented in Table 4-2. The relatively high NDF content (cell-wall constituents) of barley illustrates the contribution of hulls to the fiber content of barley. The main component of NDF in barley was hemicellulose. However, the soybean meal used in this study had a higher ADF (primarily cellulose) content than barley. The relatively high hemicellulose content of barley is reflected by the relatively high contents of arabinose and xylose. The values reported in this study were slightly higher than those reported by Henry (1985) because hydrolysis was carried out in 3 M H_2SO_4 at 110 °C for 1 h instead of 1 M H_2SO_4 at 100 °C for 1 h. Hemicellulose is composed primarily of D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose and 4-O-methyl-D-glucouronic acid (Fahey and Berger, 1988; McDonald et al., 1988; Hunt and Groff, 1990). The relatively high galactose content of soybean meal reflects the high content of raffinose and stachyose in soybean meal, 1.1 and 4.2%, respectively (Coon et al. 1990). Raffinose and stachyose are relatively high in fructose (Fahey and Berger, 1988). Soybean meal has a relatively high fructose content. However, the fructose in soybean meal was measured as mannose. The alditol acetate derivatives of fructose and mannose have identical retention times. The occurrence of N-acetylglucosamine in soybean meal likely indicates some bacterial contamination. Bacterial cell walls have a peptidoglycan framework; the polysaccharide component consists of linear chains of alternating $\beta(1\rightarrow4)$-linked N-acetylglucosamine and N-acetylmuramic acid (Voet and Voet, 1990).

The chemical composition of GAO and GUO are presented in Figures 1 and 2, respectively. Based on preliminary analysis, 1 h hydrolysis in 3 M H_2SO_4 was chosen as the optimal hydrolysis time for ingredients, diets and digesta. One hour of hydrolysis resulted in the highest release of galactose from GAO and was close to maximum release of glucose from GUO. The ratio of galactose to glucose in GAO was 2:1, indicating that the most predominant oligosaccharide was likely galactose-galactose-glucose. The GAO product contained 42.8% TOS (DM-basis). Following 1 h hydrolysis, galactose and glucose accounted for 48.2%. There was little change in the sugar composition of GAO over the period of sequential hydrolysis indicating that degradation and release rates were similar. The GAO also contained a small amount of arabinose and mannose (.31% after 1 h hydrolysis). Following 1 h hydrolysis, carbohydrates accounted for 50.2%. The GAO had a DM content of 93.5% and contained .89% N. The GAO may also have contained carbohydrates that are resistant to hydrolysis.

The predominant monosaccharides in GUO were glucose (44%) and xylose (7.6%) following 1 h hydrolysis. The GUO product contained 24.4% glucooligosaccharides (DM-basis). Therefore, other readily hydrolyzable carbohydrates that contain glucose (in addition to the glucooligosaccharides) must have been present. Mannose degradation exceeded mannose release prior to 45 min of hydrolysis. Mannose appears to be quite sensitive to hydrolysis conditions. There was little change in the sugar composition of GUO after 30 min hydrolysis. Following 1 h of hydrolysis, carbohydrates accounted for 59.7%. The GUO had a DM content of 94.4% and contained 1.33% N.

The chemical composition of the experimental diets is presented in Table 4-3. The proximate and AA composition of the experimental diets were nearly similar. The monosaccharide composition of the experimental diets reflects the substitution of cornstarch with oligosaccharides or lactitol.

The apparent ileal digestibilities of DM, CP and AA are presented in Table 4-4. The digestibilities are within the range of values reported by Sauer and Ozimek (1986). The supplementation of diets with oligosaccharides or lactitol did not affect ($P > .15$) the digestibilities of DM and CP. Of the indispensable AA, the apparent ileal digestibility of threonine in the diet supplemented with lactitol was lower ($P < .01$) than in the diet supplemented with GAO. There was a trend ($P < .10$) for the ileal digestibilities of isoleucine and lysine to be lower in the diet supplemented with lactitol than in the other diets. Of the dispensable AA, the apparent ileal digestibilities of alanine in the diets supplemented with lactitol or GUO were lower ($P < .04$) than the diet supplemented with GAO. There was a trend ($P < .07$) for the ileal digestibilities of aspartic acid and tyrosine to be lower in the diet supplemented with lactitol than in the other diets.

The apparent ileal digestiblities of CP and most AA were higher (P < .05) in period two than in period one (data not shown). These results are in agreement with those reported in Chapters two and three and by Li et al. (1993).

The monosaccharide composition of ileal digesta, as a percentage of DM, is presented in Table 4-5. The galactose concentration in digesta of pigs fed diets supplemented with lactitol was higher (P < .04) than in digesta from pigs fed diets supplemented with GAO or GUO. Once more, these results reflect the high content of galactose in lactitol. Lactitol is neither absorbed nor hydrolyzed to any significant extent by mammalian enzymes (Patil et al., 1987; Harju 1988a,b). Furthermore, there was no effect (P > .13) of diet on the monosaccharide composition of ileal digesta.

The monosaccharide composition of ileal digesta, as a percentage of total carbohydrate, is presented in Table 4-6. The predominant monosaccharides, in descending order, were glucose, xylose, arabinose and galactose, all of which are major components of hemicellulose. Galactose represented a higher (P < .04) percentage of total carbohydrate in digesta from pigs fed diets supplemented with lactitol than those supplemented with GAO or GUO.

The apparent ileal digestiblities of monosaccharides in the experimental diets are presented in Table 4-7. Supplementation of diets with GAO or lactitol decreased (P < .01) the digestibility of rhamnose. Supplementation of diets with lactitol decreased (P < .02) the apparent ileal digestibility of fucose. However, these results should be interpreted with caution. The concentrations of rhamnose and fucose in the experimental diets were relatively low, ranging from .13 to .16 and .11 to .14%, respectively. In addition, the concentrations of rhamnose and fucose in ileal digesta were relatively low, ranging from .22 to .26% and .34 to .51%, respectively. The remaining digestiblities were not affected (P > .3) by supplementation with oligosaccharides or lactitol. Low or negative digestibilities were observed for all sugars except glucose. The relatively high apparent ileal digestibility of glucose can be explained by the highly digestible nature of starch (Low, 1980; Graham, 1991). Gastrointestinal mucin is relatively high in fucose, galactose, N-acetylglucosamine and N-acetylgalactosamine (Lien and Sauer, 1993). Bacterial cell walls are another source of N-acetylglucosamine (Voet and Voet, 1990). The apparent ileal digestibilities of the aforementioned amino sugars were highly negative (< -350%). The relatively low digestiblities of arabinose, xylose, mannose, and galactose illustrate the limited amount of hemicellulose, raffinose and stachyose digestion that takes place in the stomach and small intestine. The relatively low digestibilities of these sugars and of ribose and rhamnose may be due, in part, to the relatively high content of these sugars in endogenous secretions, bacterial components, such as RNA/DNA, and non-starch polysaccharides (NSP). Similar results were reported by Vervaeke et al. (1991); the apparent ileal digestiblities of NDF, arabinose, xylose and galactose as well as total NSP (including rhamnose and mannose) in growing pigs, fed diets containing different sources of fiber, were relatively low.

The ileal output (g/d) of monosaccharides is presented in Table 4-8. The ileal output of monosaccharides was not affected (P > .3) by supplementation with oligosaccharides or lactitol.

The pH, ammonia and VFA concentrations in ileal digesta are presented in Table 4-9. The effects of supplementing diets with oligosaccharides and lactitol on pH, ammonia and VFA concentrations in ileal digesta have not been reported before in the literature. Supplementation with oligosaccharides or lactitol did not affect (P > .2) the pH, ammonia, acetate, propionate, n-butyrate, or total VFA concentrations in ileal digesta. The VFA concentrations (mol/100 mol), were also not affected (P > .9) by supplementation with oligosaccharides or lactitol.

The pH, ammonia and VFA concentrations determined in ileal digesta collected during different periods of time postprandial are presented in Table 4-10. There was no change (P > .2) in the pH of ileal digesta up to 7.5 h postprandial. The concentrations of ammonia and VFA exhibited a quadratic relationship during this time. The ammonia concentration in ileal digesta was highest (P < .03) from 0 to 1.5 h postprandial. Acetate, propionate, n-butyrate and total VFA concentrations in ileal digesta were highest ($P < .004$) in ileal digesta collected from 0 to 1.5 h postprandial. The percentage of acetate in ileal digesta was lowest ($P = .0001$) from 0 to 1.5 h postprandial. Conversely, the percentages of propionate and n-butyrate were highest ($P < .001$) from 0 to 1.5 h postprandial.

The $\log_{10}$ of the number of bacterial cells per gram of fresh ileal digesta are presented in Table 4-11. There was no effect ($P > .17$) of supplementing diets with oligosaccharides or lactitol on bacterial populations. These populations are not mutually exclusive, therefore some of the bacteria were assayed more than once. The most predominant groups were the Gram-positive anaerobes and the least predominant group were the enterococci. However, these results should be interpreted with caution due to the importance of bacteria attached to the mucosa of the stomach and small intestine, as reviewed by Conway (1989).

The effect of postprandial sampling time on the $\log_{10}$ of the number of bacterial cells per gram of fresh ileal digesta is presented in Table 4-12. The number of Gram-negative anaerobes was lower ($P < .002$) from 3 to 4.5 h than from 0 to 1.5 h postprandial. For the other classes of bacteria, there was no effect ($P > .2$) of sampling time.

In summary, the supplementation of diets with oligosaccharides or lactitol had little effect on the ileal digestibilties of nutrients. Except for galactose, supplementation of diets with oligosaccharides or lactitol did not affect (P > .13) the monosaccharide content or composition of ileal digesta. Furthermore, the output of monsaccharides in ileal digesta, pH, ammonia and VFA concentrations and the bacterial populations in ileal digesta were not affected (P > .2) by supplementation with oligosaccharides or lactitol.

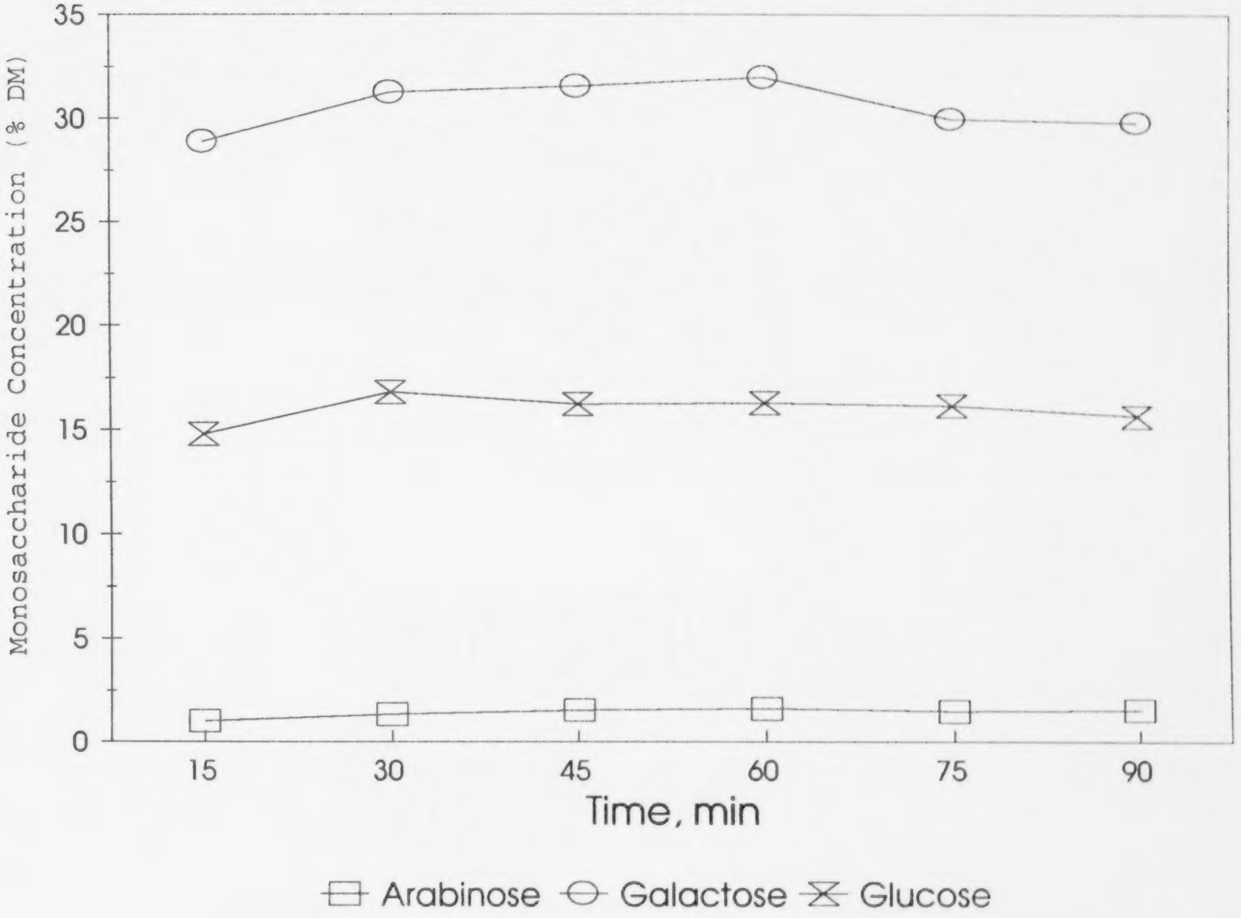

Figure 4-1. Sequential hydrolysis curves for the galactooligosaccharides following hydrolysis for different periods of time in 3 M H_2SO_4 at 110 °C. Each point is the average concentration of monosaccharides based on two determinations.

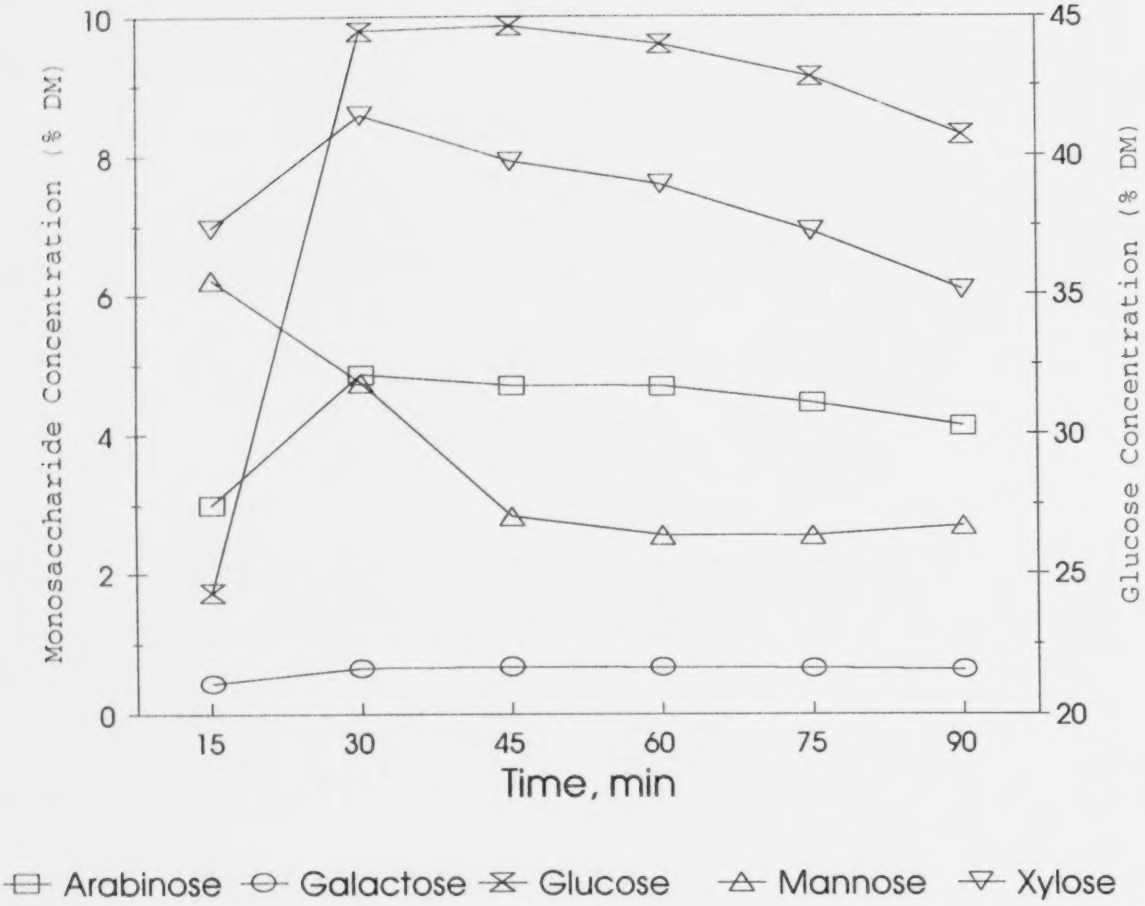

Figure 4-2. Sequential hydrolysis curves for the glucooligosaccharides following hydrolysis for different periods of time in 3 M H_2SO_4 at 110 °C. Each point is the average concentration of monosaccharides based on two determinations.

Table 4-1. Formulation (%) of the experimental diets[1]

	Diet			
Item	Control	GAO	GUO	LAC
Barley	36.30	36.30	36.30	36.30
Wheat	36.30	36.30	36.30	36.30
Soybean meal	21.00	21.00	21.00	21.00
Calcium carbonate	1.62	1.62	1.62	1.62
Monocalcium phosphate	1.44	1.44	1.44	1.44
Soybean oil	1.00	1.00	1.00	1.00
Chromic oxide	.50	.50	.50	.50
Vitamin premix[2]	.20	.20	.20	.20
Mineral premix[3]	.20	.20	.20	.20
L-Lysine (78%)	.28	.28	.28	.28
L-Threonine (98%)	.08	.08	.08	.08
DL-Methionine (98%)	.07	.07	.07	.07
Cornstarch	1.00	.50	.13	-
Galactooligosaccharides	-	.50	-	-
Glucooligosaccharides	-	-	.87	-
Lactitol	-	-	-	1.00
Total	100.00	100.00	100.00	100.00

[1] Abbreviations: GAO - galactooligosaccharides; GUO - glucooligosaccharides; LAC -lactitol.

[2] The vitamin premix provided the following per kg diet: 2,100 IU vitamin A; 240 IU vitamin D_3; 13.2 IU vitamin E; .6 mg vitamin K; 480 mg choline; 15 mg niacin; 10.8 mg d-pantothenic acid; 3.6 mg riboflavin; 1.8 mg pyridoxine; 1.2 mg thiamine; .36 mg folacin; .06 mg d-biotin; .018 mg vitamin B_{12}.

[3] The mineral premix provided the following per kg of diet: 480 mg Mg; 96 mg Fe; 96 mg Zn; 6 mg Cu; 3.6 mg Mn; .3 mg Se; .17 mg I.

Table 4-2. Proximate analysis, calcium, phosphorus, amino acid and carbohydrate content (%) of barley, wheat and soybean meal[1]

Item	Barley	Wheat	Soybean Meal
Gross energy[2]	19.22	18.44	19.27
Dry matter	86.46	86.82	87.00
Crude protein	13.44	13.81	50.47
Neutral-detergent fiber	20.66	8.15	12.75
Acid-detergent fiber	5.60	1.69	7.44
Cellulose	5.04	1.28	7.23
Hemicellulose	15.06	6.46	5.31
Ether extract	1.73	1.61	2.64
Calcium	.06	.03	.40
Phosphorus	.40	.33	.69
Ash	2.15	1.85	6.61
Amino acids:			
Indispensable			
Arginine	.56	.54	3.20
Histidine	.29	.31	1.24
Isoleucine	.47	.48	2.29
Leucine	.93	.93	3.67
Lysine	.39	.30	3.62
Phenylalanine	.71	.63	2.44
Threonine	.46	.39	1.91
Valine	.64	.56	2.29
Dispensable			
Alanine	.54	.49	2.10
Aspartic acid	.73	.65	5.55
Glutamic acid	3.49	4.36	9.37
Glycine	.55	.53	1.97
Serine	.59	.65	2.41
Tyrosine	.30	.26	1.43
Monosaccharides			
Arabinose	2.44	1.66	2.24
Fucose	tr[3]	tr	.36
Galactose	.63	.47	6.81
Glucose	66.95	74.95	12.03
Mannose	.59	.31	1.10
N-Acetylgalactosamine	tr	tr	tr
N-Acetylglucosamine	tr	tr	.16
Rhamnose	.18	tr	.23
Ribose	tr	tr	.89
Xylose	4.08	2.28	1.19
Total	74.95	79.77	25.02

[1] Dry matter basis.
[2] MJ per kilogram.
[3] Tr; present in trace amounts.

Table 4-3. Proximate analysis, calcium, phosphorus, amino acid and carbohydrate content (%) of the experimental diets[1,2]

	Diet			
Item	Control	GAO	GUO	LAC
Gross energy[3]	18.18	18.07	18.12	18.28
Dry matter	87.35	87.63	87.52	87.52
Crude protein	20.70	20.73	20.37	20.37
Neutral-detergent fiber	13.03	12.99	13.00	13.00
Acid-detergent fiber	4.18	4.17	4.17	4.17
Cellulose	3.79	3.78	3.78	3.78
Hemicellulose	8.85	8.82	8.83	8.83
Ether extract	2.68	2.72	2.61	2.55
Calcium	1.03	1.10	1.01	1.04
Phosphorus	.66	.67	.66	.67
Ash	7.26	7.31	7.29	7.15
Amino acids:				
Indispensable				
Arginine	1.06	1.09	1.07	1.05
Histidine	.48	.48	.47	.45
Isoleucine	.84	.85	.83	.82
Leucine	1.45	1.48	1.46	1.42
Lysine	1.24	1.26	1.25	1.25
Phenylalanine	1.00	1.00	1.02	.99
Threonine	.79	.81	.78	.77
Valine	.93	.93	.92	.90
Dispensable				
Alanine	.83	.84	.82	.81
Aspartic acid	1.69	1.71	1.67	1.64
Glutamic acid	4.81	4.90	4.83	4.70
Glycine	.84	.84	.83	.82
Serine	.95	1.00	.97	.94
Tyrosine	.50	.53	.52	.50
Monosaccharides[4]				
Arabinose	2.19	2.07	2.26	2.19
Fucose	.13	.12	.14	.11
Galactose	1.91	2.03	1.90	2.36
Glucose	54.40	52.57	53.31	54.59
Mannose	.57	.57	.53	.59
N-acetylgalactosamine	tr	tr	tr	tr
N-acetylglucosamine	.05	.06	.04	.06
Rhamnose	.14	.13	.16	.13
Ribose	.37	.41	.39	.36
Xylose	2.81	2.69	3.09	3.02
Total	62.57	60.63	61.83	63.40

[1] Dry matter basis.

[2] Refer to Table 4-1 for abbreviations.

[3] MJ per kilogram.

[4] Tr; present in trace amounts.

Table 4-4. Apparent ileal digestibilities (%) of dry matter, crude protein and amino acids in the experimental diets[1]

Item	Diet: Control	GAO[2]	GUO	LAC	SE[3]
Dry matter	65.4	67.4	64.9	65.9	1.40
Crude protein	72.9	75.2	71.8	71.6	1.12
Amino acids:					
Indispensable					
Arginine	82.9	84.4	83.0	82.6	.65
Histidine	80.9	81.8	80.3	79.1	.81
Isoleucine	78.8	79.6	77.8	76.6	.79
Leucine	79.4	80.0	78.7	77.6	.85
Lysine	77.4	79.0	75.5	73.7	1.37
Phenylalanine	81.4	82.3	81.4	80.4	.73
Threonine	67.0^{ab}	70.0^{a}	66.1^{ab}	63.9^{b}	1.29
Valine	76.0	76.9	75.1	73.7	.98
Dispensable					
Alanine	69.8^{ab}	71.0^{a}	67.2^{b}	65.3^{b}	1.17
Aspartic acid	72.7	74.3	71.4	70.1	1.00
Glutamic acid	87.0	88.5	87.1	86.7	.64
Glycine	64.1	67.1	63.9	65.5	2.20
Serine	74.5	77.5	75.0	74.0	1.14
Tyrosine	74.8	76.8	74.5	72.3	1.03

[1] Least squares means.
[2] Refer to Table 4-1.
[3] Standard errors of the least squares means (n = 6).
[a,b] Means within a row with different superscript letters differ (P < .05).

Table 4-5. The effect of the experimental diets on the monosaccharide content in ileal digesta[1,2]

	Diet				
Item	Control	GAO[3]	GUO	LAC	SE[4]
Arabinose	5.89	5.60	6.13	5.95	.16
Fucose	.42	.34	.46	.51	.04
Galactose	4.94[ab]	4.40[b]	4.40[b]	5.30[a]	.23
Glucose	17.52	17.12	17.48	17.13	.76
Mannose	1.58	1.53	1.58	1.52	.05
GalNAc[5]	.36	.28	.34	.39	.06
GlcNAc[6]	.87	.80	.86	.96	.05
Rhamnose	.22	.25	.25	.26	.02
Ribose	.84	.78	.77	.79	.06
Xylose	8.32	8.45	9.08	8.58	.35
Total	40.96	39.57	41.37	41.39	1.14

[1] Least squares means.

[2] Dry matter basis.

[3] Refer to Table 4-1.

[4] Standard errors of the least squares means (n = 6).

[5] N-Acetylgalactosamine.

[6] N-Acetylglucosamine.

[a,b] Means within a row with different superscript letters differ (P < .04).

Table 4-6. The effect of the experimental diets on the monosaccharide composition in ileal digesta[1,2]

	Diet				
Item	Control	GAO[3]	GUO	LAC	SE[4]
Arabinose	14.41	14.18	14.84	14.36	.41
Fucose	1.02	.88	1.13	1.23	.11
Galactose	12.03^{a}	11.10^{b}	10.62^{b}	12.82^{a}	.42
Glucose	42.74	43.21	42.20	41.35	.97
Mannose	3.86	3.89	3.85	3.68	.14
GalNAc[5]	.88	.70	.84	.94	.17
GlcNAc[6]	2.14	2.03	2.10	2.33	.15
Rhamnose	.53	.65	.62	.63	.05
Ribose	2.05	1.96	1.85	1.93	.16
Xylose	20.32	21.41	21.97	20.71	.61

[1] Least squares means.

[2] As a percentage of total carbohydrate.

[3] Refer to Table 4-1.

[4] Standard errors of the least squares means (n = 6).

[5] N-Acetylgalactosamine.

[6] N-Acetylglucosamine.

[a,b] Means within a row with different subscript letters differ ($P < .04$).

Table 4-7. Apparent ileal digestibilities (%) of monosaccharides in the experimental diets[1]

	Diet				
Item	Control	GAO[2]	GUO	LAC	SE[3]
Arabinose	6.9	6.3	4.9	3.8	5.24
Fucose	-14.8[a]	-.8[a]	-14.3[a]	-63.8[b]	13.07
Galactose	10.5	24.4	18.9	19.4	6.59
Glucose	88.9	88.7	88.5	88.9	.91
Mannose	4.8	6.4	-5.2	7.1	5.99
Rhamnose	47.1[a]	31.1[b]	45.4[a]	29.1[b]	2.99
Ribose	20.1	33.3	31.8	22.1	6.56
Xylose	-2.5	-9.1	-3.6	-.9	7.24
Total	77.3	77.4	76.5	76.8	1.54

[1] Least squares means.

[2] Refer to Table 4-1.

[3] Standard errors of the least squares means (n = 6).

[a,b] Means within a row with different superscript letters differ (P < .04).

Table 4-8. The effect of the experimental diets on ileal output[1] of dry matter and monosaccharides[2]

	Diet				
Item	Control	GAO[3]	GUO	LAC	SE[4]
Dry matter	166.52	163.55	161.05	158.50	12.37
Arabinose	9.80	9.24	9.92	9.45	.81
Fucose	.69	.59	.74	.81	.09
Galactose	8.39	7.31	7.17	8.44	.95
Glucose	29.34	28.25	28.43	27.31	2.92
Mannose	2.64	2.51	2.55	2.44	.24
GalNAc[5]	.60	.47	.56	.62	.12
GlcNAc[6]	1.46	1.31	1.36	1.52	.14
Rhamnose	.36	.41	.41	.41	.03
Ribose	1.43	1.29	1.24	1.26	.16
Xylose	13.79	13.87	14.72	13.65	1.27
Total	68.51	65.24	67.09	65.92	6.35

[1] Grams per day.

[2] Least squares means.

[3] Refer to Table 4-1.

[4] Standard errors of the least squares means (n = 6).

[5] N-Acetylgalactosamine.

[6] N-Acetylglucosamine.

Table 4-9. The effect of the experimental diets on pH, ammonia and volatile fatty acid concentrations in ileal digesta[1]

	Diet				
Item	Control	GAO[2]	GUO	LAC	SE[3]
pH	7.22	6.95	7.03	6.76	.18
Ammonia, mM	9.62	11.05	11.17	10.91	2.42
VFA, mM[4]					
Acetate	14.42	15.27	16.26	13.79	1.24
Propionate	4.41	5.13	4.99	5.27	.90
n-butyrate	1.44	1.45	1.51	1.60	.18
Total	20.28	21.84	22.76	22.67	2.07
VFA, mol/100 mol					
Acetate	73.88	72.17	73.36	70.15	2.92
Propionate	19.55	21.13	20.52	22.95	2.71
n-butyrate	6.57	6.71	6.12	6.90	.33

[1] Least squares means.
[2] Refer to Table 4-1.
[3] Standard errors of the least squares means (n = 18).
[4] Only trace amounts of isobutyrate, isovalerate, n-valerate and n-caproate were detected.

Table 4-10. The effect of sampling time on pH and volatile fatty acid concentrations in ileal digesta[1]

	Hours Postprandial			
Item	0 to 1.5	3 to 4.5	6 to 7.5	SE[2]
pH	7.05	6.87	7.03	.07
Ammonia, mM[3]	12.63[a]	9.34[b]	9.60[b]	.99
VFA, mM[4]				
Acetate[5]	18.36[a]	14.27[b]	12.74[b]	.94
Propionate[3]	7.42[a]	4.16[b]	3.56[b]	.52
n-butyrate[3]	2.42[a]	1.08[b]	1.04[b]	.16
Total[3]	28.20[a]	19.51[b]	17.34[b]	1.53
VFA, mol/100 mol				
Acetate[3]	66.53[b]	74.85[a]	75.48[a]	1.16
Propionate[3]	20.08[a]	19.61[b]	18.53[b]	1.05
n-butyrate[3]	8.39[a]	5.54[b]	5.80[b]	.24

[1] Least squares means.

[2] Standard errors of the least squares means (n = 24).

[3] Quadratic effect ($P < .02$).

[4] Only trace amounts of isobutyrate, isovalerate, n-valerate and n-caproate were detected.

[5] Linear effect ($P = .001$)

[a,b] Means within a row with different superscript letters differ ($P < .04$).

Table 4-11. The effect of the experimental diets on bacterial cell counts in ileal digesta[1,2]

	Diet				
Item	Control	GAO[3]	GUO	LAC	SE[4]
Total Lactic Acid Bacteria	9.07	9.02	8.98	9.09	.09
Homofermentative *Lactobacillus*	8.72	8.99	8.77	8.91	.13
Heterofermentative *Lactobacillus*[5]	8.37	7.45	8.30	8.47	.28
Gram-Positive Anaerobes	9.22	9.05	9.23	9.06	.10
Gram-Negative Anaerobes	7.46	7.88	7.61	7.30	.23
Escherichia coli	7.37	7.24	7.04	7.01	.23
Total Enterobacteriaceae	7.63	7.31	7.38	7.23	.27
Enterococci[6]	6.18	5.04	5.55	5.39	.43

[1] Least squares means.
[2] Log_{10} of the number of bacterial cells per gram of fresh digesta.
[3] Refer to Table 4-1.
[4] Standard errors of the least squares means (n = 12).
[5] Including *Bifidobacterium*.
[6] Genus *Enterococcus*.

Table 4-12. The effect of sampling time on bacterial cell counts in ileal digesta[1,2]

	Hours Postprandial		
Item	0 to 1.5	3 to 4.5	SE[3]
Total Lactic Acid Bacteria	8.99	9.07	.06
Homofermentative *Lactobacillus*	8.85	8.84	.06
Heterofermentative *Lactobacillus*[4]	7.95	8.22	.19
Gram-Positive Anaerobes	9.12	9.15	.06
Gram-Negative Anaerobes	7.89[a]	7.33[b]	.12
Escherichia coli	7.32	7.05	.15
Total Enterobacteriaceae	7.53	7.27	.15
Enterococci[5]	5.61	5.42	.17

[1] Least squares means.
[2] Log_{10} of the number of bacterial cells per gram of fresh digesta.
[3] Standard errors of the least squares means (n = 24).
[4] Including *Bifidobacterium*.
[5] Genus *Enterococcus*.
[a,b] Means within a row with different superscript letters differ ($P < .05$).

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

The early-weaned pig, i.e. weaned at 3 to 4 wk of age, exhibits low or no weight gain, low feed intake and often diarrhea. The supplementation of diets for early-weaned pigs with organic acids has been shown to increase ADG (Henry et al., 1985; Kirchgessner and Roth, 1990; Giesting et al., 1991) and gain to feed ratio (G/F) (Falkowski and Aherne, 1984; Burnell et al., 1988; Kirchgessner and Roth, 1987a). Supplementation with organic acids has also been shown to reduce the incidence of diarrhea after weaning (Kirchgessner and Roth, 1987a; Kirchgessner and Roth, 1990; Paullauf and Hüter, 1993). However, supplementation with organic acids may not always increase ADG or G/F (Edmonds et al., 1985; Bolduan et al., 1988; Sutton et al., 1991). As was reviewed by Kirchgessner and Roth (1988), the growth-promoting effects of organic acids may be due to: 1) improvement in the digestibility of nutrients and energy; 2) gastrointestinal effects, including antimicrobial effects in which the growth of potentially detrimental bacteria are inhibited as a result of a reduction in the gastric pH; 3) changes in intermediary metabolism, including the efficiency of energy utilization. Experiments one and two (Chapters two and three) were designed to address the first and second possible growth-promoting effects. As was reviewed by Tanksley and Knabe (1984) and Sauer and Ozimek (1986) the ileal rather than fecal analysis method, because of the modifying action of the microflora in the large intestine, should be used to determine AA digestibility.

In the first experiment semi-purified diets, which contained cornstarch and fish meal, were supplemented with 1% formic acid and contained normal or high levels of Ca^{2+} and P. There was no effect ($P > .15$) of supplementing the diets with formic acid or increasing the level of Ca^{2+} and P on the apparent ileal digestiblities of amino acids (AA). However, the apparent ileal digestibility of ash was decreased ($P < .01$) when the levels of Ca^{2+} and P were increased. This can be explained by the fact that the dietary levels of Ca^{2+} and P exceeded the absorptive capacity of transport systems, a portion of which is active, in the intestine (McDowell, 1992). With respect to the apparent digestibilities of AA, the lack of response may have been due to the highly digestible nature of the diets. Another consideration is that the response to organic acid supplementation may be different under commercial conditions, i.e. a piggery, than under more sanitary or isolated conditions as is usually the case in a metabolic trial. There was no effect ($P > .08$) of supplementing the diets with formic acid or increasing the level of Ca^{2+} and P on the pH, ammonia and VFA concentrations and bacterial populations in ileal digesta. The results of this study indicate that decreasing the pH or increasing the buffering capacity of the diet does not affect the apparent digestibility of nutrients determined at the distal ileum.

The effect of supplementation with graded levels (1.5 and 3%) of fumaric acid (FA) as well as 1.5% sodium fumarate (NaFA) on AA digestibility was investigated in experiment two (Chapter 3). Supplementation with FA or NaFA to wheat-soybean meal diets did not increase the apparent ileal digestiblities of AA. On the contrary, supplementation of 3% fumaric acid decreased (P < .04) the apparent ileal digestiblities of GE, CP, arginine, glycine and tyrosine. These decreases were linear (P < .01) for GE, CP, arginine and glycine and quadratic (P < .05) for tyrosine. These decreases may have been due, in part, to increased microbial growth and therefore biomass, in the small intestine. Several genera of enteric bacteria can reduce fumarate to succinate by fumarate reductase and this reaction is coupled to ATP synthesis (Gottschalk, 1986; Drasar, 1988). With respect to GE, another factor that may explain the decrease in digestiblities is that the absorption rate of fumarate is lower than of the products of starch hydrolysis, i.e. glucose. If this is the case, more indigestible matter would be collected at the distal ileum and therefore digestiblities would be lower. Supplementation of diets with FA or NaFA did not affect (P > .12) the pH of ileal digesta or acetate and propionate concentrations. For the diets supplemented with FA or NaFA the concentration of n-butyrate was increased (P < .05).

Supplementation of diets for early-weaned pigs with nondigestible oligosaccharides been shown to increase ADG and reduce the incidence of diarrhea following weaning (Modler et al., 1990). Similar effects were shown in calves when milk replacers were supplemented with lactitol (Nousiainen and Setala, 1990). Nondigestible oligosaccharides and lactitol are readily fermented and promote the growth of lactic acid bacteria which suppress the growth of pathogenic and putrefactive bacteria (van Velthuijsen, 1979; Tanaka et al., 1983; Hidaka et al., 1986; Modler et al., 1990; Roberfroid et al., 1993). In experiment three (Chapter four) the supplementation of diets with oligosaccharides or lactitol had little effect on the ileal digestiblities of AA or the monosaccharide content and composition of ileal digesta except for galactose which was higher ($P < .04$) in digesta of pigs fed diets supplemented with lactitol. The ileal digestibility and ileal output of monosaccharides was not affected by supplementation of diets with oligosaccharides or lactitol. The pH, ammonia and VFA concentrations and bacterial populations were also not affected ($P > .2$) by supplementation of diets with oligosaccharides or lactitol. The higher galactose content in ileal digesta was due to the presence of lactitol which indicates that a substantial proportion of lactitol is not fermented in the small intestine and will enter the large intestine. However, there was no evidence of the presence of galactooligosaccharides or glucooligosaccharides at the distal ileum. These oligosaccharides were included in the diet at a level of .2% of the diet and may have been fermented by bacteria in the small intestine. The presence of oligosaccharides in barley, wheat and soybean meal may have masked any effect of the oligosaccharides or lactitol.

Future research on the effect of supplementing diets for early-weaned pigs with organic acids is needed to improve understanding on their mode of action. More research is needed on the absorption and transport of organic acids in the intestine and their effect on microbial metabolism as well as secretions into the gastrointestinal tract. Further investigation on the effect of supplementation of organic acids on the pH in the stomach needs to be carried out. In the aforementioned studies, the lack of a response to organic acid supplementation may have been due to the fact that the diets contained adequate levels of nutrients relative to the genetic potential of the animals. If this was the case, supplementation with organic acids would not be beneficial. Future research is needed to evaluate the effect of supplementation with organic acids on nutrient digestibility when early-weaned pigs are fed diets which have a relatively low essential AA or mineral content.

Future research on the effects of supplementing diets for early-weaned pigs with oligosaccharides or lactitol is needed to improve understanding on their mode of action. Growth trials on commercial pig operations with early-weaned pigs fed diets supplemented with different levels and different types of oligosaccharides and lactitol should be carried out to determine whether or not ADG and G/F can be increased and the incidence of diarrhea decreased. Challenging early-weaned pigs with oral administration of pathogenic strains of Escherichia coli and supplementing diets with oligosaccharides or lactitol may aid in determining if the incidence of scouring can be reduced. Digestibility trials with early-weaned pigs fitted with a simple T-cannula at the distal ileum and fed semi-purified diets supplemented with increasing levels of oligosaccharides or lactitol would be useful in determining to what extent the oligosaccharides or lactitol are fermented in the small and large intestine.

In summary, these studies show that the supplementation of diets with organic acids does not increase the apparent digestibilities of amino acids and does not affect the pH, ammonia and VFA concentrations and bacterial populations in ileal digesta. In addition, supplementation of diets with oligosaccharides or lactitol had little effect on the digestiblities of nutrients, bacterial activity or bacterial populations at the distal ileum.

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